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*Chemical Toolbox for AIS Management in Hawaii:
A Review of Substances and Methods*



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Executive Summary

In response to the prominent negative impacts that aquatic invasive species (AIS) have inflicted on Hawaii's natural resources, federal and state government officials joined forces with representatives from academia, non-governmental organizations, and various industries and developed the AIS Management Plan for the State of Hawaii. Some of the specific AIS management goals established in this plan published in 2003 were 1) "the implementation of a system for rapid response to contain newly detected AIS", 2) "the integration of knowledge from efforts to both long-term containment and eradication", and 3) "the production of a review of management efforts and effective measures to be used when developing strategies and plans to address specific AIS in Hawaii". This Chemical Toolbox is a step toward fulfilling these goals. It consists of a broad literature review of chemical methods to control and eradicate AIS complemented by comparative analyses and recommendations suited for current and potential AIS problems in Hawaii.

This report is organized into six chapters. Chapter I introduces readers to the AIS problem and provides an overview of management and policy strategies for the control and eradication of AIS, in addition to a summary of pertinent legal and regulatory matters. The following three chapters (Chapters II- IV) deal with chemical substances classified according to major target AIS groups: Fish; Mollusks and other fouling animals; Aquatic plants, algae, bacteria, and viruses. Efforts to control and eradicate AIS that do not belong to any of these broad groups but that are relevant to this review (e.g. invasive crustaceans, echinoderms, fungi) are also reported and can be located by searching for their scientific or common name in this report's Species and Common Names Indexes.

Within Chapters II- IV, chemical methods with potential use in control and eradication actions are grouped into USEPA-registered active ingredients and non-registered active ingredients. Each product is reviewed for its chemical nature and suitability to control and eradicate different groups of AIS in individual chemical profiles, within each respective target group chapter.

Chemical profiles identify active ingredients' origin, toxic effects to target organisms and to non-target species, metabolic processes, pathways, and persistence in the environment, as well as common presentation and uses. This information is organized in the following subsections: Mode of action, Selectivity, Toxicology, Environmental fate and decomposition, Formulations and application methods, and Uses against unwanted AIS. Some profiles do not contain all of these subsections. That is the case of active ingredients that can be used to control more than one target group and, therefore, have more than one profile in different chapters. In these instances, information presented in preceding profiles is not repeated and instead, the reader is directed to the section which contains that information.

At the end of each chapter, active ingredients are evaluated in a comparative analysis section, with the objective of identifying which chemical methods are most appropriate to control or eradicate AIS in environments and circumstances of interest. In this analysis, I also considered data limitations, advantages, and potential side effects of active ingredients and I indicated which components are worth further investigation and perhaps the development of specific research and experiments.

In chapter V, I briefly present chemical methods that have been tested and evaluated for the control of AIS but that have been shown to be of little or no suitability for this purpose, either because they are not efficient or because they may cause unacceptable levels of undesirable effects.

Lastly, in Chapter VI, I offer an overall summary, conclusions and a list of technical and policy recommendations. There, I identify rotenone, antimycin A, acetic acid, and chlorine as promising chemical methods for some AIS management actions. Other active ingredients that merit further testing and consideration are imazapyr, hydrated lime, menadione (SeaKleen) and peracetic acid (Peraclean). I conclude the main portion of this report with a list of actions and experiments that I propose as the next step toward the establishment of an effective framework for rapid response and management of new and established AIS in Hawaii. I also identify legal and regulatory impediments that must be resolved in order to implement these actions and experiments.

While the scope of this toolbox is limited to chemical methods to control and eradicate AIS, in appendix 1, I offer a concise assessment of non-chemical methods

that are available to be used instead or in combination with chemical methods to combat aquatic invasions.

Most information presented here comes from peer reviewed papers, official government documents (e.g. USEPA Reregistration Eligibility Decisions and associated Risk Assessments and Toxicological Studies), or from reports prepared by other parties per governmental requests (e.g. studies developed by consulting agencies in lieu of governmental agencies). There are some references to electronic databases, and in these cases I cited both the date on which the information was posted (whenever available) and the most recent date on which I accessed the website. In some instances, I contacted pesticide experts, manufacturers, scientists, and managers directly and all information acquired in this way is cited in the text as personal communication.

I sincerely hope this toolbox can assist Hawaii's natural resource managers in the challenging mission of controlling, and whenever possible eradicating aquatic invasive species that threaten ecological balance in this remarkable archipelago.

Disclaimer

This report is not an endorsement of pesticide use. Chemical products, whether synthetic or organic, should be regarded as hazardous substances and as potential contaminants to the environment. Consequently, their use should be limited to instances of demonstrated need in which potential benefits outweigh potential losses and practical alternatives do not exist. Management plans and chemical applications should be developed and carried out by competent and trained professionals furnished with preliminary studies, and post-application monitoring programs.

This document has been developed to serve as a bibliographic reference tool kit for management support and has no legal or regulatory application. Policy reviews and technical analyses presented in this report represent the views of the author at the time this report was completed and shall not be quoted, referred to, or alluded to as being an official position by any government officials or institutions.

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Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

List of Acronyms and Abbreviations

a.i.	Active Ingredient
AIS	Aquatic Invasive Species
APHIS	Animal and Plant Health Inspection Service
CFR	Code of Federal Regulations
CWB	Clean Water Branch
DAR	Division of Aquatic Resources
DLNR	Department of Lands and Natural Resources (of the State of Hawaii)
DOA	Department of Agriculture (of the State of Hawaii)
DOH	Department of Health (of the State of Hawaii)
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FQPA	Food Quality Protection Act
FR	Federal Register
GUP	General Use Pesticide
IPM	Integrated Pest Management
MOE	Margin of Exposure
MP	Manufacturing-Use Product
NOAA	National Oceanic and Atmospheric Administration
OSHA	Occupational Safety and Health Administration
RED	Reregistration Eligibility Decision
RQ	Risk Quotient
RUP	Restricted Use Pesticide
SLN	Special Local Need (Registrations under Section 24(c) of FIFRA)
SMAV	Species Mean Acute Value
TGAI	Technical Grade Active Ingredient
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
USFWS	United States Fish and Wildlife Service
USGS	United States Geological Survey

Chapter I: Introduction

Overview of the Aquatic Invasive Species Problem

Aquatic invasive species (AIS) are living organisms that are non-native to a particular marine or freshwater ecosystem and whose introduction to that ecosystem causes or is likely to cause harm to human health, the environment or the economy (NISC 2006).

While in nature, all organisms migrate or disperse to some extent, current rates of human-induced invasions are estimated to be orders of magnitude greater than natural background rates (Wonham 2003). Once introduced to a new ecosystem, AIS may find suitable conditions to thrive and change the physical, chemical and biological structure of that system. AIS may prey upon and/ or compete with native species, alter nutrient availability, facilitate invasions by other AIS and significant change habitat conditions. The consequences are usually irreversible and can inflict significant impacts on the natural ecology and evolutionary processes of biological communities (HEWITT, WILLING et al. 2004; Anderson, Tan et al. 2005; Sax, Stachowicz et al. 2005).

The occurrence of AIS may also be detrimental to human health by directly triggering illnesses or by serving as vectors for pathogens (McCarthy and Khambaty 1994; Van Dolah 2000; Sandifer, Holland et al. 2004; Crowl, Crist et al. 2008). Finally, AIS may impair local and regional economies, by affecting agricultural and commercial activities (Hoagland, Anderson et al. 2002; Townsin 2003; Levin 2006; Joshi 2007). The global economic costs of AIS impacts, prevention, and control mechanisms are not well documented but are known to be quite high. AIS-related losses and damages alone cost the US at least US\$7.4 billion annually (Pimentel, Zuniga et al. 2005).

Globally, commercial shipping is the most relevant vector for AIS introductions into marine and coastal environments (Carlton, Reid et al. 1995; Carlton 1999; Bax, Williamson et al. 2003; Fofonoff, Ruiz et al. 2003). AIS are continuously transported in ballast water tanks, foul of hulls, anchor chains, and sea chests, and in this

way transferred within and among ocean basins, coastal areas, and estuaries (Carlton and Geller 1993; Wonham and Carlton 2005; Gregg, Rigby et al. 2009). Other significant vectors include intentional species introductions for commercial and biocontrol purposes (Staples and Cowie 2001), and unintentional introductions related to the transport of aquaculture gear and livestock (Naylor, Williams et al. 2001; Maki and Galatowitsch 2004) and to aquarium and ornamental trades (Padilla and Williams 2004).

The establishment and subsequent development of AIS populations involve complex processes that depend upon a convergence of factors. Both local and global conditions interfere with the success of AIS populations. At local scale, eutrophication, overfishing, interactions with other AIS and physical alteration of aquatic habitats play major roles at controlling, facilitating or exacerbating aquatic invasions (Larned and Stimson 1996; Smith, Hunter et al. 2002; Tyler, Lambrinos et al. 2007; Vermeij, Dailer et al. 2009). At global level, climate change is probably the most important environmental factor influencing aquatic invasions.

Climate change represents a new challenge for resource managers in charge of preventing, controlling, and eradicating invasive species. It affects aquatic systems by warming water temperatures, reducing ice cover, altering stream flow and ocean current patterns, modifying precipitation regimes and nitrogen distribution, increasing salinization in certain areas and carbon dioxide concentration in the water. In addition, the need for more reservoirs and canals will remove filters that currently limit the geographic range or local abundance of many invasive species. These altered conditions have profound effects on the distribution and phenology of species and on the productivity of aquatic ecosystems, which in turn may increase invasive species success in some contexts (Berge, Bjerkeng et al. 2006; Bierwagen, Rahel et al. 2008; Rahel and Olden 2008; USEPA 2008; Walther, Roques et al. 2009). For instance, changing atmospheric conditions leading to altered ultraviolet (UV) light penetration or to changing precipitation patterns can lead to altered patterns of primary production. Altered rainfall amounts could also create new patterns of estuarine salinity dynamics, favoring particular euryhaline species. Warming temperatures may lead to altered lengths of growing and reproductive seasons, or altered timing and length of feeding activity. Further, under a climatic scenario of increasing water temperatures, certain taxa at a given location may become more abundant and some may become less abundant. All of these processes could influence, in a given region, the ratios of native and introduced species, leading to altered

species interactions, and thus altered potentials for the enhancement or depression of invasions. This altered scenario, combined with changes resulting from overfishing, chemical pollution, physical destruction of natural habitats and invasion themselves, along with the unprecedented number of organisms now being moved around the globe will potentially favor a suite of species that can holistically capitalize on newly created environmental regimes (Carlton 2000).

AIS in Hawai'i

Invasive species pose a constant and significant threat to Hawaii's native ecosystems, ecosystem functions, biodiversity, watersheds, public health and industries including tourism, aquaculture and shipping (Staples and Cowie 2001; Shluker 2003). Most recent compilations for AIS in Hawaii report 301 invasive species and 117 cryptogenic species in marine and estuarine environments alone {Carlton, 2009 #548}.

While ballast water features as the most important vector for AIS introductions worldwide (Carlton, Reid et al. 1995; Miller, Andrew et al. 2004), most AIS established in Hawaii have arrived through hull fouling, solid ballast, ballast water and intentional releases, in this order (Eldredge and Carlton 2002; Godwin 2003; Carlton and Eldredge 2009).

The ecological and economic consequences of aquatic invasions in Hawaii remain largely unquantified but examples of AIS that cause negative impacts in the waters of the state abound. Hawaii hosts a number of freshwater AIS that cause significant impact to the state's natural resources. For example, several species of tilapias (e.g. *Oreochromis mossambicus*, *O. macrochir*, *Sarotherodon melanotheron*, other species and their hybrids) were intentionally introduced to Hawai'i in the 1950's for the control of aquatic weed and as baitfish but are now aquatic pests (Yamamoto and Tagawa 2000). These tilapias are voracious grazers, have high rates of reproduction success and cause substantial negative impacts on native vegetation and subsequent decline of native water birds (Englund, Arakaki et al. 2000; Staples and Cowie 2001).

In the marine environment, some prominent invasive species are the algae *Gracilaria salicornia*, *Kappaphycus* spp., *Avrainvillea amadelpha*, and *Acanthophora spicifera* which are known to displace native species and cause substantial ecological

disturbances in coastal systems, including coral smothering (Smith, Hunter et al. 2002; Conklin and Smith 2005; Schaffelke and Hewitt 2007). Other examples of nuisance marine AIS are the barnacle *Chthamalus proteus*, a common fouling organism in the high littoral zone in Hawaii that can alter natural substrates through dense colonization leading to habitat conversion and exclusion of algal grazers such as limpets (Eldredge and Smith 2001; Godwin, Rodgers et al. 2006), and the snowflake coral *Carijoa riisei* which impacts unique deep-water habitats by overgrowing endemic corals (Grigg 2003; Kahng and Grigg 2005; Kahng 2006; Carlton and Eldredge 2009).

In certain circumstances, populations of native species may also behave in an invasive manner. These phenomena are usually associated with anthropogenic disruptions in the equilibrium of the ecosystem. Disturbances such as eutrophication, overfishing, and diseases can alter the ecological balance of ecosystems and create favorable conditions for native species to undergo rapid population growth. These phenomena often induce deleterious effects similar to those caused by exotic AIS. Examples of this type of abnormal proliferation of native species are the massive blooms and coral overgrowth by *Dictyosphaeria cavernosa* in Kane'ohe Bay (Larned and Stimson 1996; Stimson, Larned et al. 2001) and by *Cladophora sericea* on Maui (Choi, Kimmerer et al. 2005). The distinction between exotic AIS and native species acting in an invasive manner is extremely important, especially in what concerns long-term management planning and prevention efforts. Nevertheless, both situations may require intervention and rapid response and the methods and techniques used to control these abrupt population outbursts tend to be similar to those used to manage AIS.

Managing AIS and Choosing Management Strategies

In this report, I follow some of the definitions presented by Wittemberg & Cock (2005) and apply the terms eradication, control, restriction and management to refer to distinct concepts. The term "eradication" refers to all efforts aimed at completely eliminating an invasive species from a given system; "control" refers to all efforts aimed at maintaining an invasive species population under a predetermined size value within a given system; "restriction" refers to all efforts aimed at containing the presence of an invasive species to a specific geographic area, and avoiding its dispersal; "management"

refers to all actions and efforts related to the eradication, control and restriction of AIS, as well as to actions related to the prevention of new introductions and re-introductions.

The successful management of AIS poses great challenges to environmental managers. While AIS eradication is the optimal management goal, it is frequently unachievable or cost-prohibitive (Mack, Simberloff et al. 2000; Bax, Carlton et al. 2001; Wittemberg and Cock 2005; Clearwater, Hickey et al. 2008). A successful invasive species policy must include strong prevention strategies to reduce the probability of new invasions as much as possible. However, because available prevention strategies are fallible, invasive species policy must also provide the means for prompt action to eradicate newly arrived but not yet established species through the application of rapid response methods. Finally, invasive species policy must address the control of established populations in an environmentally sound and safe manner.

Effective control programs typically include a suite of control activities integrating mechanical, chemical and/ or biocontrol methods. Such integrated pest management strategies allow managers to tie different control options to different areas, times, and life-history stages in an effort to minimize risks and costs while maximizing prospects for control success and protection from reinvasion.

Next, I give an example of AIS Eradication and Control Framework that can be used by managers as a model for the development of their own decision-making framework.

Proposed AIS Eradication and Control Framework

adapted from (Bax, Carlton et al. 2001; Wittemberg and Cock 2005)

Step 1: Establish the nature and magnitude of the problem by answering the following questions:

- a. Is the species really non-native?
- b. How intense is the invasion: map population size/ size of affected area.
- c. Is it causing problems (actual or potential)?
- d. How did it get there (vector)? Determine post-treatment risk of recurrence.
- e. Are there associated factors facilitating or enhancing the invasion, e.g. pollution/nutrients input, habitat alteration, overexploitation of resources, etc?
If so, a combination of control and noncontrol management actions may be required.
- f. Considering invasion and ecosystem characteristics, how much are you willing to sacrifice/ compromise in order to eradicate/ control the invasion?
Quantify direct damage.
Identify and map the area that requires eradication or control.

Assessments such as the ones required to answer the questions above are important tools to determine direct and indirect losses caused by invasions and contrast these losses against the risks involved in eradication and control measures. Although assessments are expensive, require time and expertise they are crucial to move to the following step.

Step 2: Defining goals

Stakeholders and decision makers must evaluate the assessment and define specific management goals by answering the following questions:

- a. Should all the problems and losses caused by the invasion be addressed?
- b. Is eradication a must or are control and containment actions acceptable?
- c. What are the specific legal responsibilities and impediments?
- d. What performance criteria will be used to measure intervention success?

Step 3: Consider full range of alternatives

- e. Management options include noncontrol (e.g. habitat improvement, pollution abatement), and a combination of various chemical, mechanical and biological eradication, control and containment methods. Strive for an integrated pest management approach.

(Proposed AIS Eradication and Control Framework Continued)

Step 4: Determine risks

Risk is a function of likelihood of harm and the severity of the harm that results. The goal of a successful AIS control plan is to effectively address the problems generated by the invasion while minimizing the risk of undesired outcomes.

Step 5: Reduce risk

Choose methods specific to the AIS effect(s) to be controlled:

Limit control to target species and treatment area.

Give preference to biocontrol agents that are native and chemical/physical methods that are biodegradable, nonpersistent, and reversible or that can be neutralized.

Run controlled experiments before full-scale implementation.

Establish acceptance to forfeiting certain areas or nontarget organisms.

Step 6: Assess benefits or risks of full-scale implementation

Support for control depends on a consideration of the potential benefits and negative outcomes associated to the project. This can be done by using a cost-benefit analysis that weighs the expected values from the positive outcomes from the management plan over expected value of negative impacts of the same plan plus the cost of the project. A simplified equation for this calculation is shown below:

$$\frac{\text{Invasion Magnitude (\$)} \times \text{Likelihood of successful control (P)}}{(\text{Adverse results Magnitude (\$)} \times \text{Likelihood of adverse results (P)}) + \text{Cost of control (\$)}}$$

where:

Invasion Magnitude represents the negative impacts to the economy, environment and human health associated with that invasion (quantified in monetary units). Examples would be the economic impact of an invasion on local fisheries or tourism.

(Proposed AIS Eradication and Control Framework Continued)

Likelihood of successful control is the probability P ($0 < P < 1$) that the control plan will achieve the expected result of bringing the value of *Invasion Magnitude* to zero.

Adverse results Magnitude represents the potential side-effects associated to the control methods selected in the control (quantified in monetary units). Examples would be the death of non-target organisms, or the temporary closure of a recreational area.

Likelihood of Adverse results is the probability P ($0 < P < 1$) that side-effects associated to the control plan will occur.

Cost of Control is the total amount of money that the project is expected to cost; this item should also include monitoring costs if applicable.

Step 7: Monitor the program

After the eradication or control plan has been put into action it is important to keep track of the results and potential side effects of the treatment. Methods to keep AIS populations and non-target species at check should be identified and the timeframe for adapting monitoring effort should be determined. Depending on the methods used, analysis of the water, soil or organisms may be necessary to identify post-treatment toxicological or ecological effects that may require attention. Baseline data may have to be collected prior to the implementation of the control/ eradication project.

The most difficult task associated with the use of a framework such as the one presented above is the quantification of potential and actual impacts due to invasions and treatment side-effects. When economic valuations of the resources impacted or at risk are available, this task becomes significantly easier, but these assessments are rarely on hand. For instance, in spite of the outstanding importance of Hawaiian aquatic resources for biodiversity conservation and local economies, to this date only one economic

valuation has been conducted to quantify the value of a segment of this resources (i.e. coral reefs) and it dates back to 2002 (Cesar, van Beukering et al. 2002). In most cases, managers must be able to perform some sort of cost-benefit analysis utilizing the best scientific information available, experts' opinions and parameterization.

In contrast, rapid-response actions usually require immediate intervention and the decision making process must be as simplified as possible. The development of a full cost-benefit analysis associated to the decision-making process is usually unfeasible and therefore managers must have pre-determined provisions that are acceptable for most new detections. Often, rapid-response activities take place in small infestation areas, and side-effects from eradication methods, chemical or not, are expected to be minimal.

Chemical Methods' Advantages and Hazards

Chemical methods can be valuable tools for combating invasive species. The use of toxicants or pesticides is often the most cost-effective option available for managers, and sometimes the only alternative that can actually achieve the expected control goals (Lennon, Hunn et al. 1971; Kamrin 1997; Finlayson, Schnick et al. 2000; Mack, Simberloff et al. 2000; Netherland, Getsinger et al. 2005; Poovey and Getsinger 2005; Madsen 2006). However, it is important to mind that in every instance in which toxicants or pesticides are used as a management tool, the ecology of the treated system is inevitably disrupted.

Worthwhile chemical methods to eradicate and control AIS must have properties that meet the needs of environmental managers while minimizing adverse effects. The ideal chemical method would completely kill or inactivate the target AIS population while totally degrading to harmless constituents within a short period of time. This ideal chemical method would also inflict no harm to non-target organisms, while minimizing pain and suffering for target organisms and being effective over a broad range of water quality conditions. In addition, it would be affordable, easy and safe to apply. Unfortunately, no pesticide currently available for the control of AIS meets all of these criteria. Therefore, managers must carefully balance the benefits of using a toxicant against its shortcomings and adverse effects.

Negative impacts of the use of chemicals include direct and indirect damage to non-target species and humans, the development of pest resistance to pesticides, and secondary pest outbreaks.

The potential for a pesticide to cause direct harm to non-target species is inversely related to its selectivity. In other words, if a pesticide is highly selective, it will inactivate only the target AIS and have minimum or zero negative effect on other species that may come in contact with the product and its metabolites. While a few modern pesticides have been developed to act upon specific characteristics of certain pests, most products will inevitably cause harm to non-target species, especially to those non-target species that are close relatives, ecologically or physiologically analogous to the target species.

Pest resistance to chemicals occurs when a fraction of the invasive species population possesses a genetic variation in the trait that is targeted by the pesticide of choice and this variation in trait allows that part of the population to survive the treatment. If this genetic trait is hereditarily transferable, then the next generation will be tolerant to the chemical action of that pesticide. Pest resistance to pesticides ranges from susceptibility to tolerance to total resistance, and tends to increase with specific biological qualities such as high genetic variability within populations and short generation time. Thus, pest resistance to pesticides is more common among bacteria, algae, plant and insect species than among higher animal species. Strategies to avoid the development of pest resistance to pesticides include the use of combined pesticides at one-time applications, alternation of pesticides in subsequent applications, and combination of pesticides with non-chemical control methods.

Secondary pest outbreaks occur when, upon suppression of the target invasive species population, other species populations rapidly increase in size taking over the niche that was being occupied by the target AIS. AIS management plans should consider the risk for secondary pest outbreaks. If these cannot be forecasted or prevented in the original control effort, a new control effort for that emerging invasive species may be required.

In addition, certain pesticides have the potential to persist in the environment for extended periods of time and may cause the contamination of adjacent soil, groundwater, superficial water, and air leading to indirect harm to non-target species. Depending on the chemical nature and formulation of the pesticide, and on the conditions and properties of the application site and application methods, the active ingredient may be transported in the environment through particle drift, evaporation, leaching or yet by residues on treated species. Other indirect negative effects of pesticides include the bioaccumulation of the active ingredient in the food chain, or the intoxication of non-target species by the inactive components of the end-product, such as solvents, surfactants and additives.

Finally, synthetic pesticides are relatively recent inventions, and possible adverse effects of most pesticides are not completely understood by science. This is especially true in what concerns potential long-term effects. Although it can be challenging to quantify long-term effects of stressors across temporal scales, it is

important to fully understand the impacts of toxicants on environmental health. Short-term effects are easier to assess than long-term effects, but are typically poor predictors of population level changes and are more likely to give the erroneous impression that certain toxicants are innocuous or harmful (Rohr, Sager et al. 2006).

For all the reasons outlined above, when choosing a chemical control method for AIS, managers must be able to access the best scientific information available about target species, the ecosystem to be treated and the selected treatment method(s) and consider pros and cons in face of the particular characteristics of the project. A comprehensive cost-benefit analysis should be carried out. Short and long-term risks and associated levels of uncertainty must be scrutinized and, in face of uncertainties, AIS managers must abide by the precautionary principle (Kriebel, Tickner et al. 2001; Sadeleer 2006), while striving to resolve such uncertainties.

Supplemental and alternative pest eradication and control strategies that should be considered when developing a control or rapid response plan are mechanical (e.g. traps, nets, explosives), physical (e.g. the manipulation of environmental conditions such as temperature, salinity or pH), and biological methods (e.g. the introduction of a natural predator, competitor or parasite). These methods are briefly reviewed in Appendix 1 of this Toolbox.

Also, managers should consider combining chemical applications with measures that can minimize chemical treatment side effects. For instance, in some cases, it is possible to apply neutralization agents that can change the chemical properties or structure of the pesticide, partially or completely deactivating its toxic action. Another example of mitigation measure that may be used in certain situations is the collection of non-target organisms prior to the application of a pesticide with low selectivity. The non-target organisms can be netted out and placed in holding tanks and buckets for replenishment after the toxic agent has vanished from the water body. This technique may be expensive and laborious because a significant number of specimens must be collected and maintained alive and healthy for days or weeks, but can be advantageous, especially in simple systems, such as small ponds, lakes and streams.

Finally, the choice of the application method and the utilization of containment units can significantly reduce chemical treatments' side effects. Conventional application methods include spraying (usually with backpack units, but also

from airplanes) and direct dosing of liquid or dissolved powdered pesticides into the water body with buckets, drip stations or boat dispensers. Alternative pesticide application technologies can be used to increase AIS treatment efficiency and safety and containment units can be used to restrict the pesticide action to a small volume of water contained in an area around the target species.

Pesticide Application and Containment Methods

The mode of delivery of pesticides is very important for both treatment effectiveness and selectivity. Alternative application and containment methods have the potential to significantly increase contact between the chemical and the target organisms while reducing risks to applicators and other non-target organisms. This is especially true in aquatic systems, where traditional liquid and powdered chemical formulations tend to rapidly dissolve and disperse upon contact with ambient water. As a result, managers are usually required to use higher amounts of pesticides than what would be necessary if the application could be done in a way in which the chemical presence is limited to a smaller area.

Next, I present some selected pesticide application and containment methods for the combat of AIS in aquatic environments.

BioBullets

The BioBullet technology was originally developed for the control of zebra mussel *Dreissena polymorpha* in closed water systems. The method consists of the encapsulation of an active ingredient in microscopic particles of edible material. The mussels' natural filtering ability then removes and concentrates the particles from the water, without stimulating the valve closing response. By using the mussels' filtering behavior to concentrate BioBullets, the absolute quantity of active ingredient that needs to be added to the water is expected to be reduced substantially. This approach allows for the particles to break up and dissolve completely within a few hours, thus reducing or potentially eliminating the risk of polluting the ecosystem. The effectiveness of a toxin in the control of biofouling filter-feeders is expected to be enhanced greatly by using this

technique, which has the potential to improve the control of other filter-feeding AIS (Aldrige, Elliot et al. 2006).

Active ingredients already used in BioBullets include potassium chloride, acetic acid, citric acid and calcium hydroxide. Species being targeted include zebra mussel (*Dreissena polymorpha*), sea squirt (*Didemnum vexillum*), and various species of fouling bivalves in South America (David Aldridge, Pers. Comm.).

Gel adjuvants

Various gel adjuvants have been mixed with herbicides to optimize the control of aquatic weeds in New Zealand and Australia (Chisolm, Harper et al. 2007).

Alginate gum (Torpedo[®]), guar gum (Aquagel[®], Hydrogel[®]) and methocel (hydroxypropyl methylcellulose, Depth Charge[®]) are all gel adjuvants formulated to mix with an active ingredient, usually Diquat. The most widely used gel adjuvant is Aquagel, marketed as Hydrogel[®] in Australia (Chisolm, Harper et al. 2007).

Guar gum is a non-toxic polysaccharide starch, which can be mixed on site to obtain any desired viscosity. It is superior to alginate gum, as it retains a consistent viscosity at any temperature. Guar gum is currently listed by the USEPA as an “Inert Ingredient of Minimal Concern”, thus it is exempt from federal registration under section 25(b) of FIFRA (List 4A, Cas# 9000-30-0). The state of Hawaii (DOA) also exempts all ingredients listed in List 4A from state registration.

Guar gum, in the Hydrogel composition, is heavier than water and sinks when applied to lakes, ponds and watercourses. It penetrates thermal layers and is less subject to drift than ordinary water based herbicides. It sticks to submerged aquatic plants, what means that the active ingredient, which is added to Hydrogel prior to application remains in the target zone, ensuring optimum herbicidal activity. Hydrogel producers claim that the product reduces the amount of herbicide to be used as much as 80% compared with conventional application techniques. They also claim that, if used as recommended, the product breaks down rapidly in natural water, presenting no hazard to fish and other non-target life (Bhula 2008).

Hydrogel is most effective when applied into clean water and should not be applied when suspended organic matter is over 1ppm. Best results are said to be achieved when treating lakes and ponds under calm conditions, and the time of year has less influence on efficiency than weather conditions at the time of spraying. Application of Hydrogel can be done from a knapsack, gun and hose, boat-mounted boom or helicopter-mounted boom. Because the viscosity of Hydrogel may vary with temperature, it is important to calibrate application equipment before each use, at least until experience has been built up in the use of this product (Bhula 2008).

The differential response observed in some submerged plants could be related to less retention of Hydrogel on certain morphological types, such as the fan-like Cabomba leaves (Chisolm, Harper et al. 2007).

Vessel Wrapping System (IMProtector™)

The IMProtector™ is a technology developed by Aquenal Ltda. to reduce the risk of introduction and translocation of marine pests in biofouling on small vessels. It is a system designed to isolate and decontaminate the hulls of vessels up to 25m in length without requiring their removal from the water. It can be used to rapidly isolate a vessel with a fouled hull while it is at anchor, on a mooring or alongside a jetty or pontoon or as a control tool in the event of a new infestation to reduce the risk of translocation by hull fouling. It could also become part of “responsible cruising” for yacht owners to use before sailing from infested ports to pristine coasts (Shields and Coutts 2009).

The standard IMProtector is a heavy duty, fiber reinforced PVC sheath 15 m long and 3 m deep with inflatable floatation collars. A 20 m long and 3 to 5 m deep version has been developed for larger vessels. The 15 by 3 m IMProtector weighs approximately 100 kg. According to the manufacturer, the whole apparatus can be lifted by 3 men and carried folded in a small trailer, the back of a station wagon or a small boat, and can be deployed by two people from the vessel being quarantined, a small dinghy or a marina pontoon. Enclosing and isolating the vessel takes about 15 minutes, and tying off and pumping out the IMProtector takes about another 30 minutes (Shields and Coutts 2009).

Decontamination of fouled hulls using the IMProtector is said to be possible with or without chemicals. The “set-n-forget” treatment consists of leaving the hull

wrapped until the water that is enclosed between the sheath and the hull becomes anoxic, effectively suffocating some of the fouling organisms. This has been applied with some success in eradication and control attempts in New Zealand (Coutts and Forrest 2007). The thick black membrane blocks out light preventing photosynthesis and is effectively impermeable to dissolved oxygen. Dissolved oxygen levels have been measured to fall to less than 1% within 22 hours of completion of pumping and all organisms appeared dead within 7 days. This treatment inactivates the majority of organisms, particularly mobile fauna, within 24 hours. However, this strategy does not kill all fouling organisms. For instance, some mature mussels have been found to survive for up to 12 days in anoxic conditions (Shields and Coutts 2009).

Ongoing experiments are testing the acceleration properties of various combination treatments with mild chemicals, such as chlorine and acetic acid. These products have been shown to increase mortality rates in treatments lasting only a few hours (Coutts and Forrest 2007). It is envisaged that a volume of water containing these treatments will be circulated within the IMProtector for the period required to achieve complete mortality then it will be pumped back to a holding tank before the IMProtector is removed. Aquenal Ltda. is also testing the effect of elevating the concentration of carbon dioxide which has been shown to render mussels more susceptible to subsequent treatment with chlorinated water (Shields and Coutts 2009).

Custom-made containment units

The invasive marine alga *Caulerpa taxifolia* was discovered into a southern California lagoon, Agua Hedionda, and a small embayment, Huntington Harbor, in June of 2000. One of the few examples of successful AIS rapid response followed by eradication was initiated promptly after this discovery. Field containment and treatments began 17 days after the discovery of the invasive algae (Anderson 2005).

A coalition of state, federal and local agencies as well as private groups and non-governmental organizations was formed (Southern California Caulerpa Action Team) and concluded that the best approach for treatment of the *C. taxifolia* colonies was the construction of small polyvinyl chloride (PVC) frames that were to be placed over the plants and then covered with black 20 mil PVC sheeting for containment of chemical (Woodfield and Merkel 2006).

The sizes of the tarps used in this eradication project ranged from 500 m² to cover the few large colonies initially discovered, to about 1 m² to cover small plants found in later surveys. The sides of the tarps were anchored and sealed to the bottom with gravel filled bags. An overhang was provided between the edge of the colony and edge of the bagged area to ensure that a margin of uninfected area was also covered and treated. Initially, liquid sodium hypochlorite (ca. 12% stock solution) was injected into the tarped areas via ports in the PVC tarps fitted with caps. Smaller colonies were later covered with the PVC tarps without a frame, beneath which several 2.5 cm diameter solid chlorine-releasing tablets were placed. The tablets were much easier for scuba divers to handle and required far less equipment than was required for injecting liquid sodium hypochlorite (Anderson 2005).

The success of the treatment has been confirmed by subsequent monitoring of treated area (Anderson, Tan et al. 2005; Woodfield and Merkel 2006) and by a study of the fate of *C. taxifolia* fragments (Williams and Schroeder 2004).

Regulatory and Legal Considerations

It is of utmost importance that, when developing and implementing AIS control, eradication or rapid response plans, managers understand and comply with all the legal and regulatory aspects of such undertakings. A number of activities that may be considered as part of AIS management plans (e.g. the use of chemicals and mechanical methods) are regulated by legal instruments at national, state and local levels. Legal procedures, matters of jurisdictions and authority and the need for permits and licenses from various agencies in addition to compliance with their standards and procedures must be observed.

Here, I offer a brief overview of the national and state laws and regulations that apply to the control of AIS in Hawaii, with special focus on the legal aspects of using chemical control methods in aquatic environments. Some international agreements that deal with prevention and management of biological invasions also exist, but these fall outside of the scope of this report. Note that legal procedures at state level vary among states, thus the information on state laws and regulations presented here apply to the

state of Hawaii only. Moreover, laws and regulations are constantly being challenged, revised and amended, thus it is essential that managers consult with federal and state authorities for updates.

Aquatic invasions are complex by nature. They usually involve a variety of interconnected environments, species, human activities and industries, and this complexity is reflected on the myriad of legal instruments that apply to the management of AIS. For the purposes of developing an AIS management plan that includes the application of chemicals to aquatic environments, one can classify the pertinent legal instruments into three groups: 1) those that deal with the management of AIS in general, 2) those that regulate the use of pesticides and 3) those that apply to the control of water pollution.

General AIS Laws and Regulations

Federal Level

Legal consideration to the need for prevention and management of nonnative species in the US dates back to the beginning of the 20th century with the promulgation of the Lacey Act of 1900 (16 USC). Since then, several federal acts that deal directly or indirectly with the prevention, control and mitigation of nonnative bioinvasions have been enacted and amended, giving various degrees of regulatory authority to a number of agencies and departments and composing what some consider a rather paradoxical and fragmented legal arrangement (Miller, Andrew et al. 2004). Indeed, legal authorities pertaining to the management of invasive species in the US are available to at least three federal departments under over twenty different federal acts. For example, the Department of Agriculture (USDA) has legal authorities over certain invasive species matters under the Plant Protection Act, the Federal Seed Act, the Animal Quarantine Laws and the Federal Noxious Weed Act, among other acts. The Department of Interior holds authority over certain invasive species matters through the Lacey Act, the Endangered Species Act, the Nonindigenous Aquatic Nuisance Prevention and Control Act (NANPCA, as amended by the National Invasive Species Act, NISA). The Department

of Commerce has AIS authorities through NANPCA, the Magnuson-Stevens Fishery Conservation and Management Act, and the Coastal Zone Management Act (NISC 2001).

Of all the above-mentioned acts, NANPCA is certainly the most encompassing and relevant legal instrument for the purposes of the present review. NANPCA was enacted in 1990 with the main function of establishing a federal program to “prevent the introduction of, and to control the spread of introduced aquatic nuisance species”. It was a first formal attempt to tie the many aspects of AIS management under a single umbrella policy and to set the framework for a comprehensive AIS program. NANPCA was later amended by the National Invasive Species Act (NISA) of 1996. NANPCA/ NISA included the establishment of a Federal Aquatic Nuisance Species Task Force (ANS Task Force), and called for the development of State and regional management plans to assist in the control of AIS. The Task Force consists of ten federal agency representatives and twelve Ex-officio members, and is co-chaired by the United States Fish and Wild Life Service (USFWS) and The National Oceanic and Atmospheric Administration (NOAA). The Task Force coordinates governmental efforts dealing with AIS in the US with those of the private sector and other North American interests via regional panels, committees and work groups (<http://www.anstaskforce.gov/default.php>).

Another legal instrument relevant for this discussion is the Executive Order (EO) 13112, signed by President Clinton in 1999. Although executive orders cannot create new government powers, they can direct federal agencies to act in particular policy directions. EO 13112 was intended to provide a more cohesive and integrated approach to the invasive species management problem, and created the National Invasive Species Council (NISC).

NISC is an inter-Departmental council that helps to coordinate and ensure complementary, cost-efficient and effective federal activities regarding invasive species. NISC members include three co-chairs, the secretaries of the Agriculture, Commerce, Interior, and the secretaries of State, Defense, Homeland Security, Treasury, Transportation, Health and Human Services, as well as the Administrators of the Environmental Protection Agency, the US Agency for International Development, the US Trade Representative, and the National Aeronautics and Space Administration. NISC was required to produce a National Invasive Species Management Plan to encourage planning and action at the local, tribal, State, regional, and ecosystem-based levels. NISC

also develop recommendations for international cooperation, provide guidance on incorporating prevention and control of invasive species into the National Environmental Policy Act (NEPA), facilitate development of a communication network to document, evaluate, and monitor impacts from invasive species on the economy, the environment, and human health, and initiate the development of an information-sharing system that facilitates the exchange of information concerning invasive species.

State Level

In Hawai'i, the Department of Agriculture (DOA) and the Department of Land and Natural Resources (DLNR) have primary authority over many aspects related to AIS. The DOA primarily administers prevention efforts to minimize terrestrial and aquatic invasive species introductions through the regulation of plants, non-domestic animals and microorganisms' importations. Their activities include the issuance of permits, and implementation of quarantine and inspection programs in accordance with their mandate, as established by HRS §150A. The DLNR is legislatively authorized to manage the aquatic resources of the state and is responsible for conserving, protecting, and enhancing the state's aquatic renewable resources and habitats. It does so mainly through the Division of Aquatic Resources (DAR). The DAR exercises its statutory authority over the regulation of industries and activities that may introduce AIS such as aquaculture, aquatic pet, trade and aquaria and commercial and recreational shipping as mandated in HRS §187A-6.7 and §187A-31&32. The DAR also develops research and programs for the conservation and recuperation of aquatic resources which may include the management of AIS.

In addition, the director of the Department of Health (DOH) is mandated to "prevent, control and abate water pollution in the state" (HRS §342D-4). According to the definitions established by state legislation, "water pollution means (1) such contamination or other alteration of the (...) biological properties of any state waters (...), or (2) such discharge of any (...) substances into any state waters, as will or is likely to create a nuisance or render such waters unreasonably harmful, detrimental or injurious to public health, safety, or welfare, including harm, detriment or injury to (...) fish and aquatic life and wildlife, recreational purposes agricultural and industrial research and scientific uses of such waters (...)" (HRS §342D-1). While historically, DOH has not been as involved in

responding to bioinvasions as the DOA and DLNR have, its shared AIS-related responsibilities are unambiguous.

Two coordinating bodies have been created to address AIS matters in Hawaii: the Coordinating Group on Alien Pest Species (CGAPS) and the Hawai'i Invasive Species Council (HISC). CGAPS was formed in 1994 with participants from government and non-government agencies. HISC was created in 2002 and given statutory authority in 2003, in recognition of the ongoing need for cabinet-level participation and leadership in invasive species issues. HISC is co-chaired by the DLNR and the DOA. Members of HISC include the University of Hawaii, Hawaii Department of Business, Economic Development and Tourism, Hawaii Department of Health, Hawaii Department of Transportation, County Mayors, Hawaii Department of Defense, federal agency representatives and Non-profit agency representatives. HISC was established for the special purpose of providing policy level direction, coordination, and planning among state departments, federal agencies, and international and local initiatives for the control and eradication of harmful invasive species infestations throughout the State of Hawaii and for preventing the introduction of other invasive species that may be potentially harmful (HRS 194-2).

Under NANPCA/ NISA, state governors are authorized to submit comprehensive AIS management plans to the Task Force for approval and qualify for federal technical and financial assistance. The State of Hawaii's AIS Management Plan (Shluker 2003) was finalized in September of 2003.

Pesticide Laws

Federal Level

The Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), as amended by the Federal Environmental Pesticide Control Act of 1972 (FEPCA), is the principal law regulating pesticides in the US.

FIFRA is administered by the USEPA and this agency is responsible for the control of the manufacture, registration, and labeling of pesticides in the US. Under

FIFRA, all pesticides sold, distributed or applied in the country must be registered by the USEPA, except for products classified as “minimum risk pesticides”. Minimum risk pesticides are those in which all active-ingredients meet certain pre-established criteria and are exempt from federal registration under section 25(b) of FIFRA. Castor oil, cedar oil, citronella, peppermint, sodium chloride, soybean are a few examples of active-ingredients currently exempted from USEPA registration under 25(b) of FIFRA. The USEPA does not review or register pesticides that satisfy the 25(b) criteria, and in Hawaii, these products are also exempt from state registration (USEPA 2009).

FIFRA's registration process for pesticides is based on scientific studies that show that, if used in accordance with their labels, the approved pesticides do not pose unreasonable risks to people or the environment. Also, this law requires all pesticides that were registered before November 1, 1984 to be reregistered to ensure that they meet today's more stringent standards. In evaluating pesticides for registration and reregistration, the USEPA requests and reviews studies from manufacturers, describing the human health and environmental effects of each active ingredient that composes the end-use product. During this process, the USEPA must also comply with the requirements under the United States Federal Food, Drug, and Cosmetic Act of 1938 (FFDCA), and the Food Quality Protection Act of 1996 (FQPA). At the end of the review process, the USEPA decides on whether or not to register the active ingredient, and classifies it as a General Use Pesticide (i.e. one that can be sold, purchased and applied by any person) or as a Restricted Use Pesticide (i.e. one that can only be sold, purchased and applied by authorized individuals; see below). The USEPA also determines the safety requirements that must appear on the pesticide label.

According to pesticide laws, the product label of a registered pesticide is a legally binding document. Thus, the instructions presented on the label must be followed closely. The use of a pesticide in a way that is inconsistent with its label is a violation of FIFRA and can result in civil or even criminal action. One important caveat related to pesticides labels is that the use of a registered product to combat a pest that is not listed on the product label is permitted under Section 2(ee) as long as the application is done to a use site that is listed on the product's label.

FIFRA requires all persons who apply RUP pesticides to be either certified according to the provisions of that act, or to work under the direct supervision of a certified

applicator. Commercial, private and public applicators must demonstrate a practical knowledge of the principles and practices of pest control and safe use of pesticides. In addition, applicators using or supervising the use of any restricted use pesticides purposefully applied to standing or running water are required to pass an exam to demonstrate competency as described in the Code of Federal Regulations (40 CFR 171.4). See below for more information on the procedures to become certified pesticide applicator in Hawaii.

State Level

States regulate pesticides under both FIFRA and state pesticide laws, which can be more but not less restrictive than FIFRA. In Hawaii, the Hawaii Pesticides Law (HRS§ 149A) is the main instrument for the registration, licensing, certification, recordkeeping, usage and other activities related to the safe and efficacious use of pesticides. The Hawaii Pesticides Law is administered and enforced by the Division of Plant Industry within DOA through their Pesticides Administrative Rules (HAR§ 4-66).

The DOA is primarily concerned with the sale, distribution, use and disposal of pesticides in the state, and all pesticides sold and applied in the state must be licensed by the DOA. In other words, a pesticide may be FIFRA-registered to be used in the US, but it can only be used in Hawaii if it is also registered by the DOA. A list of currently DOA registered pesticides can be found at their website (<http://hawaii.gov/hdoa/pi/pest/SLN.pdf>).

The procedures to receive and maintain a DOA authorization to sell and store RUPs in Hawaii are described in HAR §4-66-51 and 52, and involve acquiring a 5-year renewable license and submitting yearly reports of the dealer's activities. The DOA also administers the certification process for RUP applicators in accordance to the conditions established in HAR§4-66-(56 through 62). The DOA offers exams for RUP applicator licenses every other week (DOA 2009). The Cooperative Extension Service, a part of the College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, periodically offers courses aimed at reducing risks associated with the handling and application of pesticides and preparing applicants for DOA's certification exam for RUPs (Nagamine 2009).

What is FIFRA's relevance to AIS management?

AIS management actions that rely on the application of pesticides must comply with FIFRA and the regulations promulgated thereunder. If a pesticide is already registered and labeled by the USEPA for the control and eradication of species in the aquatic application sites of interest, the action does not require additional permitting under FIFRA (although it may require an NPDES and state permits; see below for more information on this topic).

If the rapid response or control action requires the use of an unregistered pesticide or a pesticide registered for a different end use or use pattern, then two sections of FIFRA may apply: sections 18 and 24(c).

Section 18 of FIFRA authorizes the USEPA to allow states to use a pesticide for an unregistered use for a limited time (no longer than 1 year) if the USEPA determines that emergency conditions exist. An emergency condition is an urgent, no routine situation that requires the use of a pesticide or pesticides and meets the following criteria: 1) no effective registered pesticides are available; 2) no feasible alternative control practices are available; 3) the situation involves the introduction of a new pest, will cause significant economic loss, or will present significant risks to human health, endangered species, or the environment. Detection of an AIS can qualify as an emergency condition. Natural resource managers considering use of an unregistered pesticide or a pesticide registered for a different end use or use pattern to eradicate or control AIS should consult their lead state agency for pesticides about the possibility of developing a Section 18 emergency exemption application (USEPA 2005).

Most requests for Section 18 emergency exemptions are made by state lead agricultural agencies (in Hawaii that is the DOA), although the USDA and the USDI can also request exemptions. Requests are most often made for pesticides that have other similar uses registered. The state agency evaluates the requests and submits requests to USEPA for emergency exemptions they believe are warranted. During a period of approximately 50 days of receipt, the USEPA performs a multi-disciplinary risk assessment of the requested use, relying largely on data that have already been reviewed for the pesticide. A dietary risk assessment, an occupational risk assessment, an ecological and environmental risk assessment, and an assessment of the emergency are conducted prior to making a decision. For the past several years, USEPA has also

evaluated the risk to the most sensitive sub-population (often infants and children) in its dietary risk assessments. The Agency's evaluation also includes an assessment of the progress toward registration for the use in question.

There are several categories of emergency exemptions under Section 18: 1) specific exemptions are issued to avert significant economic loss or a significant risk to endangered or threatened species, beneficial organisms, or the environment; 2) quarantine exemptions are issued to control the introduction or spread of a new or currently localized pest; 3) public health exemptions are issued to control a pest that poses a significant risk to human health; 4) crisis exemptions are issued in instances when the time between discovery of the emergency and the time when pesticide use is needed is insufficient to allow for the authorization of a specific, quarantine, or public health exemption. Quarantine exemptions are generally the most appropriate for AIS rapid response and control actions (USEPA 2005).

If a need is immediate, a state agency may issue a crisis exemption which allows the unregistered use for 15 days. The state notifies USEPA of this action prior to issuing the exemption, and the USEPA performs a cursory review of the use to ensure there are no concerns. If concerns are noted, the USEPA confers with the state, and under extreme cases may not allow a crisis to be declared. If the state follows up the crisis with, or has already submitted, an emergency exemption request, the use may continue under the crisis until the USEPA has made a decision on the request. If the state does not also submit an emergency exemption request, the USEPA must still establish the appropriate tolerance(s) for the crisis use.

Under Section 24(c) of FIFRA, states have authority to add uses to pesticides based on special local needs. Different from the Section 18 Emergency Exemption, states may not register new active ingredients under Section 24(c), only new use sites and patterns. Section 24(c) registrations are also referred to as state labels or special local need (SLN) registrations and are considered Federal registrations authorizing distribution and use within the granting state only.

State registrations under Section 24(c) are subject to USEPA's regulations at 40 CFR Part 162. To be eligible for a Section 24(c) Registration for AIS management purposes, states must show that all of the following conditions exist: 1) there is a SLN need for the use within the state, which may be an existing or imminent pest problem

within a state for which the state has determined that an appropriate federally-registered pesticide is not sufficiently available; 2) registration for the same use has not previously been denied, disapproved, suspended or canceled by EPA, or voluntarily canceled by the registrant subsequent to EPA issuing a notice of intent to cancel that registration because of health or environmental concerns, unless such denial, disapproval, suspension or cancellation has been superseded by a subsequent EPA action; 3) the registration is in accord with the purposes of FIFRA.

Each state designs its own review process and timeline for state pesticide registration. If 1) the pesticide's composition is not similar to any Federally-registered pesticide, 2) the use of the pesticide is not similar to any Federally-registered use of the same pesticide or a pesticide of similar composition, or 3) USEPA has denied, disapproved, suspended, or canceled registration of other uses of the same pesticide or uses of pesticides of similar composition, then the state is required to conduct an ecological risk assessment (ERA) to determine if the pesticide will cause unreasonable adverse effects on humans or the environment.

All products registered by a state must meet all appropriate packaging standards and might need to be classified as restricted use if their toxicity exceeds USEPA's specific hazard criteria. Depending on the length of time needed to conduct an ERA, Section 24(c) pesticide registrations requiring an ERA may be more useful for ongoing control of AIS rather than for carrying out AIS rapid response actions (USEPA 2005). If a state decides to issue a Section 24(c) registration, it must send EPA a notification package within 10 days of issuing a registration. The USEPA has 90 days to verify that the special local need registration meets FIFRA requirements. If the USEPA subsequently disapproves the registration, sales and distribution must stop immediately.

Lastly, an important section of FIFRA for the purposes of building AIS management capacity is Section 5 on Experimental Use Permits (EUP). The USEPA may grant an EUP to researchers wishing to gather data necessary to grant registration under Section 3 of FIFRA for a pesticide not registered with the Agency, or a new use of a registered pesticide. The USEPA does not require an EUP when experimental work is limited to laboratory or greenhouse tests and the researcher neither intends nor confers pest control benefit to those conducting it. Also, the USEPA has determined that an EUP is not required for limited field tests involving use of a particular experimental compound if

the tests satisfy all of the following conditions: 1) tests are conducted on a total of not more than one surface-acre of water; 2) if testing for multiple target pest species at the same time and in the same locality, the one surface-acre limitation encompasses all target pest species; 3) the waters involved in or affected by the tests will not be used for irrigation, drinking water supplies or body-contact recreational activities; and 4) tests are not conducted in waters which contain or affect any fish, shellfish, other animals, or plants taken for recreation or feed unless an appropriate tolerance or exemption from a tolerance has been established.

Water Pollution Control Laws

Federal Level

In 1972, the US Congress passed the Federal Water Pollution Control Act (FWPCA) which, with subsequent amendments, is commonly referred to as the Clean Water Act (CWA) (P.L. 92-500). The preamble to the CWA states that the goal of the Act is to ensure that the nation's waters are "fishable and swimmable." The 1987 Federal Water Quality Act Amendments (P.L. 100-4) placed new emphasis on nonpoint source pollution management and contained specific requirements and responsibilities for state pollution control programs, including submittal of a Nonpoint Source Assessment Report and a Management Plan to the USEPA for approval.

Two Sections of the CWA deserve mention here: Sections 401 and 402. Section 401 (State Certification) of the CWA (33 U.S.C. §1251 et seq) Title IV (Permits and Licenses) requires that any applicant for a federal license or permit to conduct any activity which may result in any discharge into navigable waters, shall provide the licensing or permitting agency (in this case the USEPA) with a certification from the state in which the discharge originates or will originate that any such discharge will comply with state effluent limitations and water quality standards promulgated in accordance with other sections of the CWA.

Importantly, if the state denies the 401 certification, the denial acts as an absolute veto of the federal license or permit application. The state denial is not reviewable by the permitting or licensing agency or by the federal courts. The state

decision is thus reviewable through state courts (33 USC § 1341(a)(1)). The 401 State Certification requirement with respect to an application for a federal license such as a NPDES permit shall be waived upon a written notification from the state that it expressly waives its authority to act on a request for certification, or a written notification from the licensing or permitting agency to the Regional Administrator of the failure of the state to act on such request for certification within a reasonable period of time (maximum of one year) after receipt of such request, as determined by the licensing or permitting agency (40 CFR 121.16).

Section 402 (Use of Chemicals and Toxic Compounds) of the CWA establishes the National Pollutant Discharge Elimination System (NPDES) permit program to regulate point source pollution into waters of the US. This section states specific discharge limits, and monitoring and reporting requirements, as well as special conditions. The USEPA is charged with administering the NPDES permit program, but can authorize states to assume many of the permitting, administrative, and enforcement responsibilities in its lieu. Authorized states are prohibited from adopting less stringent standards than those established by the NPDES, but may adopt more stringent standards if allowed under state law. All new permit applications are then submitted to the state agency for NPDES permit issuance. In Hawaii, the Department of Health (DOH) is in charge of the 401 Certification and administers the NPDES permitting process in lieu of the USEPA.

The Coastal Zone Act Reauthorization Amendments of 1990 required Hawaii, as one of the states with a federally-approved coastal zone management (CZM) program to develop and implement a coastal nonpoint pollution control program, to be approved by the NOAA and by the USEPA. State programs must be developed jointly by the coastal zone management agency (Hawaii Department of Business, Economic Development and Tourism) and the water quality agency (Hawaii Department of Health, DOH).

State Level

Hawaii's Department of Health (DOH) is responsible for controlling water pollution in the state. DOH's authority over the application of chemical products to water bodies is mandated in Chapter 342D (Water Pollution) of the Hawaii Revised Statutes

(HRS). According to it, the DOH is the administrator of that Chapter, and has as duties to “prevent, control and abate water pollution in the State” (HRS, §342D-4).

The DOH’s director establishes by rule, water quality standards, effluent standards, treatment and pretreatment standards, and standards of performance for specific areas and types of discharges in the control of water pollution, thereby allowing for varying local conditions (HRS,§342D-5). Under the public trust doctrine, the DOH must issue permits and ensure that the prescribed measures are actually being implemented after a thorough assessment of the possible adverse impacts the development would have on the state’s natural resources; this duty is consistent with the constitutional mandate under article XI, §1 of the Hawaii constitution and the duties imposed upon the DOH by this chapter and chapter 342E (111 H. 205, 140 P.3d 985).

The definition of water pollution under state law is broad enough to include any substance that will “change the physical, chemical, or biological properties of any state waters”; and the definition of State waters include “all waters, fresh, brackish, or salt, around or within the State, including but not limited to coastal waters (i.e. up to 3m of the coast line), streams, rivers, drainage ditches, ponds, reservoirs, canals, ground waters, and lakes; provided that drainage ditches, ponds, and reservoirs required as a part of a water pollution control system are excluded” (HRS §342D-1).

To give effect to this mandate, the DOH has promulgated two Administrative Rules: HAR 11-54 (Water Quality Standards), and the HAR 11-55 (Water Pollution Control).

The Water Quality Standards consists of the general water quality antidegradation policy. It specifies designed uses, classifies State waters, and sets the water quality certification system, criteria and standards. This HAR Chapter is commonly referred to as the “Section 401”, in reference to Section 401 of the CWA (33 USC§1251), explained in the above subsection. This common reference to Section 401 may seem to imply that the existence of the state water quality certification is only necessary if a federal license is required, but that is not the case. The Hawaii State Law vests the DOH with the authority to embargo and impose penalties to any project involving discharges into state waters that has not received a state permit, are not in accordance with the state law, or has not been given a “Variance”(§342D-7), regardless of any federal requirements.

The other legal instrument with which the DOH exercises its statutory authority of water quality issues is the Water Pollution Control Chapter 55 of Title 11 (HAR 11-55). This Chapter focuses on the administration of the National Pollutant Discharge Elimination System (NPDES) permits (33 USC§1251). In Hawaii, the DOH administers the NPDES permitting, i.e. it has to make sure all the projects that will incur discharges into water bodies are in compliance with HAR11-54, HAR 11-55, and ultimately HRS 342D; as well as with all federal regulations and standards.

Does CWA Section 402 apply to AIS rapid response or control actions?

An interpretive statement issued by USEPA in January 2005 established that, if consistent with all relevant requirements under FIFRA, the application of a pesticide to waters of the US did not constitute the discharge of a pollutant and consequently did not require a federal NPDES permit in the following two circumstances: 1) the application of pesticides directly to waters of the United States to control pests (e.g. applications to control mosquito larvae, aquatic weeds, or other pests that are present in the waters of the United States); 2) the application of pesticides to control pests that are present over waters of the United States, including near such waters; that results in a portion of the pesticides being deposited to those waters (e.g. the aerial application of pesticides to waters of the United States or of insecticides to a forest canopy where waters of the United States may be present below the canopy, or applications of pesticides over or near water for control of adult mosquitoes or other pests.).

A decision by the United States Court of Appeals for the Ninth Circuit (*Fairhurst vs. Hagener*) reaffirmed EPA's decision that a pesticide applied to a river for the purpose of "eliminating pestilent fish species is not a pollutant for the purposes of the Clean Water Act (...)and thus not subject to the Act's permit requirements." On October 22, 2007, the DOH incorporated USEPA's interpretation of the CWA into Hawaii its administrative rules. The amended "Water Pollution Control Chapter" (HAR 11-55-04 (h)) reads exactly as the USEPA's interpretative statement. That means that the state agreed that a NPDES permit and associated Section 401 certification would no longer be required for AIS chemical control activities, as long as 1) the pesticide was registered under FIFRA (both at federal and state levels), 2) the applicator was certified by the DOA

if the pesticide is a RUP, and 3) applications were done in conformity with the product's label. Nevertheless, at the time this regulatory change occurred, the DOH failed to modify its "Water Quality Standards Chapter" (HAR, 11-54) accordingly. Although the "Water Quality Standards Chapter" does not specifically address pesticides, it addresses biocides under its "Basic Water quality criteria applicable to all waters". HAR 11-54-4(a)(4) states that "(a) All waters shall be free of substances attributable to domestic, industrial, or other controllable sources of pollutants, including (...) (4) High or low temperatures, biocides, pathogenic organisms; toxic, radioactive, corrosive, or other deleterious substance at levels or in combinations sufficient to be toxic or harmful to human, animal, plant, or aquatic life, or in amounts sufficient to interfere with any beneficial use of the water (...)". Therefore, in spite of DOH's clear intent to be consistent with the then current federal regulation, the application of aquatic pesticides for AIS combat remained illegal in Hawaii due to the regulatory contradiction between HARs 11-54 and 11-55.

A recent court decision, however, rested on deciding that pesticides, even those that are in compliance with FIFRA, can be "pollutants" within the meaning of the CWA (See 33 U.S.C. 1362(6)). On January 7, 2009, the U.S. Court of Appeals for the Sixth Circuit decided that the USEPA's rule exempting the application of pesticides from the NPDES permit requirement contravene the "clear and unambiguous" language of the Clean Water Act (*National Cotton Council of America et al. v. United States environmental Protection Agency*).

The Court examined the meaning of "chemical wastes" and "biological material" which are defined by the CWA as "pollutants." The USEPA argued that pesticides are not "waste" when they are purposefully applied in, on or near water, thus they do not require a NPDES permit. The USEPA also argued that it should not treat biological pesticides any differently, so they, too, should be considered not to be pollutants. The Court held that while chemical pesticides that are applied and leave no residue or waste probably do not require a NPDES permit, any application of a chemical pesticide that produces a residue or a waste, requires a permit and that this was clearly what Congress intended when it passed the CWA. The court's decision also left no doubt that any biological pesticide required a permit, since Congress did not qualify "biological materials" with the word "waste".

As the court pointed out in its decision, the USEPA could issue a general permit for the application of pesticides and it would have almost the same effect as its rule exempting the application of pesticides from obtaining a NPDES permit. In fact, two states have already issued “general permits” for the application of pesticides, Washington State and California. According to the court decision, these general permits “greatly reduce [the] administrative burden by authorizing discharges from a category of point sources within a specified geographic area” and “once [the] EPA or a state agency issues such a [general] permit, covered entities, in some cases, need take no further action to achieve compliance with the NPDES besides adhering to the permit conditions.”

On April 9, 2009, the USEPA filed a motion to stay issuance of the Court's mandate for two years to provide USEPA time to develop, propose and issue a final NPDES general permit for pesticide applications, for States to develop permits, and to provide outreach and education to the regulated community. The two-year stay of the mandate was granted by the U.S. Sixth Circuit Court of Appeals on June 8, 2009.

The USEPA plans, before the ruling takes effect (on April 9, 2011), to issue a final general NPDES permit for covered pesticide applications and to assist states to develop their NPDES permits. The USEPA intends to “work closely with state water permitting programs, the regulated community and environmental organizations in developing a general permit that is protective of the environment and public health”. NPDES permits will be required for pesticides applied directly to water to control pests and/or applied to control pests that are present in or over, including near waters. Irrigation return flows and agricultural runoff will not require NPDES permits as they are specifically exempted from CWA permitting obligations.

In summary, as of today, NPDES permits are not required for the application of FIFRA-registered aquatic pesticides for AIS control in waters of the US, but will be required after April of 2011. General NPDES permits are expected to be issued to cover various aquatic pesticides at the discretion of the DOH. Nevertheless, due to the regulatory contradiction between HARs 11-54 and 11-55, the lawfulness of aquatic pesticides applications to state waters in Hawaii remains disputable.

Other Pertinent Laws and Regulations

The Endangered Species Act requires federal agencies to ensure that their actions are not likely to jeopardize listed species or adversely modify designated critical habitat. A primary use of aquatic pesticides is to eliminate invasive or non-native species in designated critical habitat so threatened or indigenous species may later be restored.

The USEPA has developed the Endangered Species Protection Program to identify pesticides whose use may cause adverse impacts on federally listed endangered and threatened species, and to implement mitigation measures that address these impacts. To assess the potential of registered pesticide uses that may affect any particular species, this agency puts basic toxicity and exposure data developed for the (re)registration decisions into context for individual listed species and considers ecological parameters, pesticide use information, the geographic relationship between specific pesticide uses and species locations and biological requirements and behavioral aspects of the particular species. A determination that there is a likelihood of potential effects to a listed species may result in limitations on the use of the pesticide, other measures to mitigate any potential effects, and/or consultations with the USFWS or National Marine Fisheries Service, as necessary. If the USEPA determines use of an aquatic pesticide “may affect” listed species or their designated critical habitat, it employs the provisions in the Services regulations (50 CFR Part 402).

Finally, compliance with the National Environmental Policy Act (NEPA) is required if federal funds are used for the control program, or if the program is authorized by a federal agency. This legislation dictates that control methods used at public facilities must not negatively affect native biota or existing water quality, and a protocol for compliance with the NEPA should be observed. An overview of the NEPA planning process can be found at <http://www.epa.gov/Compliance/basics/nepa.html>. A similar requirement exists for State actions, under the Hawaii Revised Statutes, Chapter 343 (Environmental Impact Statements).

Chapter II: Piscicides

Fisheries managers rely on a variety of tools to control and eradicate undesirable fish populations, including chemical, physical and mechanical methods. These methods can be used alone or combined in order to increased management efficiency. Nevertheless, it is a consensus among experts that other than total and prolonged dewatering, chemical treatment is the only method that can completely eliminate fish from a body of water (Meronek, Bouchard et al. 1996; Finlayson, Schnick et al. 2000; Ling 2003).

Historically, piscicides (i.e., fish toxicants) were mainly used to control out-of-balance or unwanted fish populations so that sport fish could be stocked for recreational purposes. Today, piscicides are used for a variety of reasons, including eradication of nonnative and invasive fish amid restoration projects and as a sampling tool to quantify fishes (Finlayson, Schnick et al. 2000).

Beginning in the late 1930's, various synthetic pesticides (e.g., toxaphene, dichlorvos, endrin, malathion) were developed and widely used as fish toxicants despite various negative aspects, such as high persistency in the environment and risks to human health and wildlife. As general awareness about the risks associated to synthetic pesticides grew, various regulations started to be established to limit pesticide applications and by the 1960's, the identification and the development of organic alternatives to control fishes had become urgent.

Organic compounds with piscicidal properties (e.g., rotenone, antimycin A, saponins) often serve as alternatives to synthetic pesticides. Most natural products tend to rapidly break down in the environment and are easily metabolized by animals receiving sub-lethal doses (Ling 2003). Nevertheless, it is important to be attentive to potential negative effects associated with uses of natural products as well. Depending on the compound and the circumstances of the application, natural products can be highly toxic to humans and other non-target organisms; some may bioaccumulate, and alter water quality parameters in ways that can be extremely deleterious to ecosystems, thus the

need to develop environmental and human health risk assessments for any toxicant and their end-use products, regardless of their synthetic or organic nature.

In Hawaii, various species of introduced fish cause negative impacts to indigenous species and ecosystems (Englund, Arakaki et al. 2000). Yamamoto & Tagawa (2000) list more than 35 species of fishes that have been introduced to Hawaiian freshwater systems. For instance, introduced guppies (*Poecilia reticulata*), topminnows (*Limia vittata*) and swordtails (*Xiphophorus helleri*) have been identified as carriers of parasitic nematodes and tapeworms that infect native gobies, 'O'opu (Yamamoto and Tagawa 2000). Western mosquito fish (*Gambusia affinis*) preys upon eggs, larvae, and fry of sport fish and native fish in areas outside of their native habitat (Courtenay and Meffe 1989). Tilapias (various taxa) are responsible for outcompeting desirable fish species, pushing endemic species, such as the endemic anchialine pool shrimp 'Ōpae 'ula (*Halocaridina rubra*) toward extinction (Brock and Kam 1997) and consuming virtually all available aquatic vegetation and invertebrate resources in wetland areas, becoming thus, a major cause for the decline of Hawaiian water birds (Englund, Arakaki et al. 2000).

In the next section, I first review the two only USEPA-registered general piscicides and their neutralization process. Then, I consider some non-registered compounds with piscicidal properties of interest and conclude this chapter with a comparative analysis that evaluates the advantages and disadvantages of these active ingredients and their relevance for the control AIS in Hawaii.

USEPA-registered Piscicides

Of all the substances known to have piscicidal properties, only four chemical products are currently registered by the USEPA for piscicidal uses in waters of the US: TFM (3-trifluoromethyl-4-nitrophenol) and niclosamide, which are registered lampricides, and antimycin A and rotenone, which are general piscicides.

TFM is currently used as the primary tool to control sea lamprey in tributaries of the Great Lakes. Niclosamide is usually applied in combination with TFM to sample lamprey prior to treatment with TFM or where TFM alone is ineffective or too expensive, such as in deep or turbid waters. Niclosamide is also registered by the USEPA as a molluscicide, and is used for the control of snails.

Although some non-target aquatic species may be negatively impacted by lampricide applications, these pesticides are not expected to control fish species other than sea lampreys (USEPA 2006). Because sea lamprey invasions are not likely to occur in Hawaii, lampricides are considered to fall outside the scope of this report. Therefore, TFM is not reviewed here and niclosamide is reviewed for its molluscicidal properties in Chapter III. Information about sea lamprey control and TFM can be found at the Great Lakes Fishery Commission's webpage (www.glfc.org) and in McDonald & Kolar (2007).

Antimycin A

General information

Antimycin A is a naturally occurring substance extracted from cultures of actinobacteria of the genus *Streptomyces*. It exhibits antifungal, insecticidal and mitocidal properties, in addition to exceptional piscicidal properties (Hamilton, Carroll et al. 1969).

Antimycin A has been used over the past decade to restore US federally-listed threatened and endangered fish to their native habitats, being applied to ponds, lakes, rivers and streams. Antimycin A is most frequently used in recreational fishing and aquaculture industries to remove scaled fish from catfish fingerling ponds.

Antimycin A is non-repellent to fish. This characteristic has been identified as an advantage that would enable effective control of target fish in the upper layer of thermally stratified lakes or in littoral zones without harm to non-target fish in deep strata or in open water (Lennon, Hunn et al. 1971).

Antimycin A was first isolated in 1945 and registered as a fish toxicant in 1960. Its reregistration was completed by the USEPA in 2007. To give effect to antimycin A's reregistration, the manufacturer must yet amend the product's label to include, among other provisions: 1) the prohibition of antimycin A's application to marine or estuarine environments, 2) the mandatory deactivation of treatment outflow with potassium permanganate, 3) the prohibition of the harvesting of surviving fish from selective kill for 12 subsequent months, 4) a maximum application rate of 25 ppb, and 5) the prohibition of public access to the treated site for seven days after an application (USEPA 2007).

Mode of action

Antimycin A uncouples oxidative phosphorylation by blocking the electron transport pathway to Complex III within the mitochondria, ultimately causing death by oxygen deprivation (Tzung, Kim et al. 2001), in a process similar to the one provoked by rotenone (Singer and Ramsay 1994). However, different from the effect of rotenone, the action of antimycin A in fish is irreversible, i.e., once fish are exposed to effective doses, they will not recover even if placed in clean water (Chapman, Fairchild et al. 2003).

Selectivity

At lower rates (5-10 ppb), antimycin A acts as a selective piscicide, eliminating some fish including salmonids, but not others such as catfish (order Siluriformes), shortnose gar (*Lepisosteus platostomus*), bowfin (*Amia calva*) and goldfish (*Carassius auratus auratus*). At higher rates (15-25 ppb), antimycin A causes complete fish kill (USEPA 2007).

Toxicology

Antimycin A is classified by the USEPA as a Restricted Use Pesticide (RUP) due to its aquatic toxicity and the need for highly specialized applicator training to minimize human exposure (USEPA 2007). The USEPA has recently approved antimycin A's reregistration process. Despite discrepancies on toxicological and metabolism studies, the agency concluded that human health and ecological risks could be reduced beyond their level of concern upon the minimization of human and other non-target organisms' exposure to the product (USEPA 2007).

The USEPA concluded that antimycin A is not a dermal irritant and eye irritation was resolved within 48 hours following exposure (USEPA 2006). However, label amendments for antimycin A prohibit the use of treated water for swimming, drinking or irrigation until measured antimycin A residues drop below $0.015 \mu\text{g L}^{-1}$. The potential effects of consuming fish killed by antimycin A are not completely understood, thus all fish killed by antimycin A must be collected and buried. Harvesting of surviving fish after a selective kill in aquaculture ponds must be precluded for 12 months.

There are no data available for chronic toxicity. A 90-day subchronic rat study resulted in a lowest-observed-adverse-effect level of $0.5 \text{ mg Kgday}^{-1}$. A no-observable-adverse-effect level was not established and no other relevant adverse effects were observed. There are no data for reproductive, developmental, mutagenicity, nor carcinogenicity effects of antimycin A.

At concentrations used to control pest fish populations, antimycin A has been considered to have minimal effects on other aquatic organisms

(Finlayson, Schnick et al. 2002). For instance, Chandler & Marking (1979) found that antimycin A was less toxic to the Asiatic clam (*Corbicula manilensis*) than to fish. Fish and other aquatic organisms have also been found to be more sensitive to antimycin than mammals and birds, owing to the chemical's rapid absorption into the bloodstream from the water across the gills (Finlayson, Schnick et al. 2002).

Direct effects of antimycin A applications on terrestrial plants and animals are expected to be minimal. Antimycin A may be ingested by certain types of birds and mammals in drinking water or as residues in aquatic food sources. Birds, mammals, and reptiles could also be exposed through dermal contact while in treated waters. For many such species, these types of exposure would be transient. It is assumed that species exposed frequently, such as piscivorous birds, ducks, muskrats, garter snakes, and others, would be most at risk from the use of antimycin A and that the risk from transient exposure would be relatively insignificant (Turner, Jacobson et al. 2007).

Environmental fate and decomposition

Due to antimycin A's recent advent and limited usage, environmental persistence and toxicological data are scarce. Models indicate that antimycin A is not likely to persist in the environment, nor to bioaccumulate and its low vapor pressure and low Henry's Law constant limit its volatility (USEPA 2007).

Some studies suggest that hydrolysis is the primary route of antimycin A degradation (Walker, Lennon et al. 1964; Lee, Derse et al. 1971), but aerobic processes may also interfere in antimycin A's metabolism (USEPA 2006). Degradation products are believed to include blastmycic acid, antimycin lactone, and antimycic acid (Walker, Lennon et al. 1964), but there is no quantitative information regarding the relative concentrations, and other metabolites may exist (USEPA 2007).

In studies conducted by the USEPA, antimycin A degraded rapidly upon contact with water under static conditions ($t_{1/2} < 12$ hours at pH values ranging from 1 to 9). However, an aerobic aquatic metabolism study requested by the USEPA amid Antimycin A's reregistration process indicated that, at pH 6.5, antimycin A degrades to half-life in 23 to 47 days. This substantially longer half-life associated to aerobic processes adds uncertainty to the data from hydrolysis studies. It is speculated that the longer half-life registered by this study could be due to the fact that these experiments included sediment, and thus antimycin A could have adsorbed to sediment particles (USEPA 2006). Although sorption studies for antimycin A are not available, once the compound is bound to sediment it is

not expected to be bioavailable and any amount of antimycin that may desorb is expected to degrade rapidly given its short half-life.

Antimycin A' degradation rate has been described as inversely related to pH, but observed values have differed greatly among studies (Lee, Derse et al. 1971) and recent observations indicate the relationship may not actually exist (USEPA 2006).

Formulations and application methods

The only antimycin A end-use product currently registered for sale is Fintrol™ which is solely produced by Aquabiotics Corp., a small company based in Bainbridge Is., WA, USA. This is a liquid concentrate that after dissolved in water can be applied by a drip-feed device as part of a drip station or with a backpack sprayer, boat bailer, or other hand-held sprayer.

Antimycin-impregnated baits have been tested against common carp (Rach et al. 1994). The bait pellets consisted of fish meal, a binding agent, antimycin and water. Baits dosed with 10 mg of antimycin A per g caused low (19%) to high (74%) mortalities in fish feeding voluntarily on 50 g of the toxic bait in each of three earthen ponds. These authors concluded that baits are best used in conjunction with other management techniques and should be distributed only when feeding aggregations of the target species and low numbers of non-target species are present.

Fintrol is not currently licensed for use in the State of Hawaii (HI Dept.Ag. 2008) and its production is currently under a halt. Fintrol's sole producer has said to be looking for a new supplier and does not know when that will happen (Mary Romeo, President of Aquabiotics Corp., Pers. Comm.).

Use against unwanted fish

Antimycin has been successfully used to eradicate several non-native fish populations in the Us and in other countries. Brook trout (*Salvelinus fontinalis*), for example, was eradicated from a tributary of the Yellowstone Lake, US, with antimycin A (Gressell 1991). The treatment covered the drainage of the tributary and included a 23.6-ha lagoon. Post-treatment surveys revealed that spawning migrations of the desirable Yellowstone cutthroat trout (*Oncorhynchus clarkii bouveri*) could still occur. Treatment success appeared to be a result of accurate estimation of toxin dispersal and good application techniques (Gressell 1991).

Antimycin A has also been used to eradicate or reduce pest fish populations in Scottish lochs and streams. The piscicide has generally been found to

be less toxic to bottom-dwelling invertebrates than to fishes (Finlayson, Schnick et al. 2002).

Antimycin treatment was used to remove nonnative mosquito fish (*Gambusia affinis*) from a brook in Arizona because they were competing with the endangered local species, the Gila topminnow (*Poeciliopsis occidentalis*). The mosquito fish appeared to have been completely removed and the replacement populations of topminnows rapidly expanded. Several months later, specimens of this AIS were found in the system again, but managers believe this was caused by reintroductions (Meffe 1983).

Rotenone

General information

Rotenone is a substance that has piscicidal, insecticidal and some acaricidal properties. It is extracted from the roots, fruits and flowers of various tropical and subtropical plant species, the most common sources being the roots of plants of the *Derris*, *Lonchocarpus*, and *Tephrosia* genera (USEPA 2007).

Rotenone has been used for centuries by indigenous tribes of various parts of the world, mainly as narcotics to capture fish for eating purposes. For instance, the utilization of pounded roots of *Tephrosia purpurea* (syn. *T. piscatoria*; 'auhuhu in the Hawaiian language) as a fishing tool in Samoa and in the Hawaiian Islands and the poisonous properties of the leaves, stems, seeds and roots of this species are well documented in the literature (Stokes 1921; Irvine and Freyre 1959; Tabrah and Eveleth 1966; Cox 1979).

In the US, rotenone has been used as a fisheries management tool since the 1930's (Finlayson, Schnick et al. 2000) and currently, there are at least ten different rotenone-based end-use products being commercialized in this country.

Rotenone was first registered in the US in 1947 as a piscicide and insecticide for crops and residential applications. It was reregistered by the USEPA in 2007, and as part of the reregistration process, the USEPA reviewed the toxicity and environmental fate of rotenone and scrutinized the risks associated with different uses and application methods. Amid this process, registrants filed to cancel registrations for all non-piscicidal uses of rotenone.

Rotenone is most often used to control undesired fish in standing water, such as large lakes and reservoirs, but also in rivers and streams. It has

been identified as an important fish sampling tool in coastal environments (Robertson and Smith-Vaniz 2008). However, due to the lack of toxicological studies that characterize long-term effects of rotenone to marine ecosystems, the USEPA has banned the application of rotenone to marine or estuarine environments; the prohibition will be explicitly stated in rotenone-products' new label. Other relevant changes in the upcoming label include: 1) the mandatory deactivation of rotenone with potassium permanganate, 2) the establishment of a 200 ppm maximum application rate, 3) the verification of drinking water intake to ensure that residual rotenone concentration is below 40 ppb, and 4) the recommendation that fish killed by the treatment is collected and buried (USEPA 2007).

Mode of action

Rotenone interrupts cellular respiration through uncoupling oxidative phosphorylation within the cell mitochondria by blocking electron transport at complex I (Fajt and Grizzle 1998; Ling 2003). It inhibits the oxidation of NADH to NAD, blocking the oxidation by NAD of substrates such as glutamate, alpha-ketoglutarate, and pyruvate. This blockage is overcome by Vitamin K3 (menadione sodium bisulphite), which apparently activates a bypass of the rotenone sensitive site (Singer and Ramsay 1994).

Selectivity

At high concentration (~100-200 ppb), rotenone acts as a broad-spectrum pesticide affecting all aquatic fauna, including amphibians and invertebrates (Skaar 2001, Lennon 1971, Schnick 1974b). At lower concentration, rotenone is somewhat selective; it kills certain fish such as rainbow trout (*Oncorhynchus mykiss*), bluegill sunfish (*Lepomis macrochirus*) but not others like zebrafish (*Danio rerio*) and black bullhead (*Ameiurus melas*) (Finlayson, Schnick et al. 2000; USEPA 2006).

Toxicology

Rotenone is registered as a Restricted Use Pesticide (RUP) due to acute inhalation and acute oral toxicity and due to toxicity to fish and other aquatic organisms (USEPA 2007).

Rotenone end-use products are of moderate toxicity to mammals and avian species (Finlayson, Schnick et al. 2000; Ling 2003; Kegley, Hill et al. 2007; USEPA 2007). Human poisoning is more likely to occur as a result of inhalation rather than ingestion. Symptoms of non-lethal intoxication such as headaches, sore throats, sores on mucous membranes, skin rashes and

severe irritation of the eyes have been reported in humans following prolonged occupational exposure to rotenone dusts, thus applicators should be properly qualified and wear appropriate protective gear (USEPA 2006).

There is no evidence that piscicidal applications of rotenone have caused birth defects, reproductive dysfunctions or cancer in animals. Chronic systemic exposure of lab rats to rotenone via brain injections has been linked to Parkinson's disease-like symptoms {Betarbet, 2000 #97; Sherer, 2003 #561}. In response, registrants filed to cancel reregistration of all rotenone uses other than piscicidal ones, based on the logic that chronic systemic exposure to rotenone would be of particular concern for dust products when used in agricultural and residential settings, but not for controlled piscicidal uses (USEPA 2006).

Environmental fate and decomposition

Hydrolysis and photolysis appear to be the primary routes of rotenone breakdown, but biodegradation cannot be ruled out. Rotenone is highly degradable particularly in warm ($\geq 25^{\circ}\text{C}$), clear, alkaline waters ($\text{pH} > 9$) and under full exposure to light. The breakdown of rotenone during the summertime may occur so rapidly due to concomitant high light exposure and high water temperature that this may cause difficulties during the application. Unless all parts of the water body are treated simultaneously, fish may migrate back into previously treated waters in which the concentration of the active ingredient are already too low to be efficient (Ling 2003). Higher pH and temperature seem to reduce rotenone's half-life in warm waters, but aquatic field studies have shown that rotenone can persist in cold water at sufficiently high concentration to cause fish mortality for at least 25 days, even in alkaline conditions (USEPA 2006).

Rotenone is not expected to leach, contaminate ground water, nor bioaccumulate in the food web and usually breaks down completely within hours to several weeks (Cheng, Yamamoto et al. 1972; Gilderhus, Allen et al. 1986; Dawson, Gingerrich et al. 1991; Draper 2002; USEPA 2006). Data analyzed by the USEPA (USEPA 2006) indicates that rotenone bonds to sediment with sufficient strength and is unlikely to leach in most circumstances, the exception being in very sandy soils with low organic carbon content. The potential of rotenone to leach and contaminate groundwater is thus small, apparently not related to temperature or pH, and ultimately limited by its fairly rapid hydrolysis rate. The primary first-order decomposition product of rotenone is rotelanone (Cheng, Yamamoto et al. 1972; Newsome and Shields 1980; USEPA 2006), a substance reported as less toxic than rotenone (Soloway 1976) but somewhat more persistent in the environment (USEPA 2006). Faster neutralization by oxidation occurs

when rotenone is mixed with potassium permanganate (Engstrom-Heg and Colesante 1979; Finlayson, Schnick et al. 2000).

Formulations and application methods

There are currently two approved formulations for rotenone products: liquid and wettable powder. Based on the USEPA's risk estimates, liquid formulations result in lower occupational exposure and are more compatible with existing closed system technologies than wettable powder products. Also, liquid formulations are more stable, keeping their piscicidal properties for longer while on storage, and are more effectively dispersed in water. On the other hand, most liquid preparations are petroleum-based thus more expensive, and flammable. Also, they produce noticeable tastes and odors in treated waters, thus are easily detected and avoided by fish, while wettable powder formulations apparently contain fewer potentially toxic impurities and inert ingredients that may pose risk to human health and to the environment (Turner, Jacobson et al. 2007).

Indeed, a recurring concern regarding the use of rotenone-based piscicides is the toxicity of other ingredients that compose rotenone end-use products, specifically those used as surfactants, synergists (e.g. piperonyl butoxide) and solvents (e.g. xylenes, ethyl benzene, acetone, naphthalene, 1,2,4-trimethylbenzene). A relatively new rotenone product, CFT Legumine, is currently the most promising formulation in the market. According to its manufacturer (CWE Properties Ltd., LLC), CFT Legumine is a 5% rotenone product which contains less petroleum hydrocarbon solvents in its composition, thus resulting in fewer residual products and lower environmental risks.

Liquid formulations may be applied using a boom or other mechanized equipment that releases the product below the water's surface, or with aircraft, backpack sprayer or other hand-held nozzle to release the product above the water's surface. Wettable powder formulations may be applied with the same above-described methods, except for the backpack sprayer, which is prohibited to reduce applicator's exposure (USEPA 2007).

Currently, there is only one rotenone end-use product registered for piscicidal applications in Hawaii, Prentiss Prenfish Toxicant (EPA Registration #655-422), a 5% rotenone liquid emulsifiable pesticide. This product's state registration was due to expire on December 31st of 2009.

Use against unwanted fish

Rotenone has been extensively used to control and eradicate exotic and invasive fishes in freshwater systems in several states and in other countries.

In California, white bass (*Morone chrysops*) and northern pike (*Esox lucius*) were successfully eradicated from reservoirs with the use of rotenone (McClay 2000). In Australia, the product has been used to control isolated populations of carp (*Cyprinus carpio*) (Barnham 1998). In New Zealand, mosquito fish (*Gambusia* spp.) and koi carp (*Cyprinus carpio*) were eradicated with rotenone from artificial ponds in order to prevent their spread into larger natural waterways (Shaw and Studholme 2001; Dean 2003).

Rotenone was regularly applied to kill invasive fish in anchialine ponds in the island of Hawaii (Hualalai Resort) in the years 2007 and 2008 at least (Chai and Mokiao-Lee 2008).

Post-treatment Neutralization of Antimycin A and Rotenone

The neutralization of outflow from water bodies treated with rotenone and antimycin A using potassium permanganate (KMnO₄) will become a mandatory practice as established by these products' post-reregistration labels (USEPA 2007; USEPA 2007).

Potassium permanganate is one of the most widely used inorganic chemicals for the treatment of municipal drinking and wastewater. Drinking water treatment plants use this chemical to oxidize iron, manganese, and arsenic to remove color, and to treat for biofilm in raw water intake pipes. Potassium permanganate is also used in fish farms to prevent or alleviate oxygen shortages in rearing ponds. The chemical works by oxidizing decaying plant matter and other organics so that they consume less oxygen, thereby relieving oxygen depletions that otherwise could result in fish kills (Archer 2001).

Monitoring data indicate that deactivation of rotenone by potassium permanganate can be effective. Water temperatures below 10 °C (50 °F) can result in longer detoxification lag time. In laboratory setting, potassium permanganate reduced the half-life of antimycin from hours to 7- 11 minutes (Archer 2001).

Potassium permanganate can be highly toxic to freshwater fish and its toxicity is inversely related to temperature. The amount and duration of use depends on a number of environmental factors and the quantity of toxicant to be deactivated. In the laboratory, exposure to 2 mg.L⁻¹ of KMnO₄ was lethal to rainbow trout (*Oncorhynchus mykiss*) within hours (Archer 2001). When applied at 1.5 mg.L⁻¹ in the absence of readily oxidizable substances, potassium permanganate achieved lethality in westslope cutthroat trout (*Oncorhynchus clarki lewisi*) after 16 to 24 hours of exposure (Turner, Jacobson et al. 2007).

Potassium permanganate is quickly broken down when it reacts with organic material, with antimycin A or with rotenone in water. Breakdown components of potassium permanganate (i.e. potassium, manganese, and water) are common in nature and have no known deleterious environmental effects at concentrations used for neutralization of piscicides (Finlayson, Schnick et al. 2000).

Since high dosages of permanganate may be toxic to aquatic organisms, detoxification procedures should utilize calibrated equipment to achieve minimum effective concentration of permanganate to neutralize the piscicide. The concentration of both the pesticide and the neutralization compound can be monitored with the utilization of analytical chemistry techniques, such as HPLC (high performance liquid chromatography). Alternatively, monitoring stations consisting of caged live fish can be placed at the downstream limit of the treatment area to verify detoxification of the piscicide and potassium permanganate.

Sodium permanganate (NaMnO_2) has also been used for the neutralization of rotenone and antimycin A. As potassium permanganate, sodium permanganate is a strong oxidizing agent that has short lifetime in the environment and is readily degraded by organic matter and inorganic oxidation substances. The breakdown components of sodium permanganate are sodium, manganese, and water. Sodium permanganate is also used for chemical oxidation of chlorinated organic contaminants in soil and groundwater. Liquid sodium permanganate is injected into the soil or groundwater and allowed to disperse through the contaminated media (Archer 2001). Sodium permanganate, however, has not been included as an alternative for potassium permanganate in the amended labels for rotenone and antimycin A's end-use products.

Selected Non-registered Piscicides

Ammonium compounds and derivatives

General information

Ammonia and its derivatives are present in the environment as a result of biological activity mainly as byproducts of natural physiological processes and decomposition, and also due to industrial activity such as intensive farming. Ammonia is the main final product of protein metabolism and a major excretory product of fishes.

Products containing unionized ammonia (i.e. anhydrous ammonia, NH_3) and the ammonium ion (NH_4^+) have been tested and used for piscicidal purposes (Boyd and Tucker 1998), but these compounds are not currently approved for piscicidal use in the US.

The maximum limits for ammonia and ammonium ion concentration in waters of the state are set by the Water Quality Standards Rules for the

State of Hawaii (HAR 11-54). For instance, HAR 11-54 establishes the following ammonia standards: in Pearl Harbor, geometric mean to not be exceeded of 10 µg per liter; in all other estuaries geometric mean to not be exceeded of 6 µg per liter; most embayments, geometric mean to not be exceeded of 3.5 to 6 µg per liter; most coastal waters geometric mean to not be exceeded of 2 to 3.5 µg per liter.

Mode of action

Ammonia is highly toxic for aquatic organisms including fish. Elevated concentrations of the ammonium ion (NH_4^+) within the bodies of fish and other vertebrates interferes with osmoregulation at the gills and disrupts blood chemistry, causing convulsions and death (Eddy 2005).

Selectivity

Ammonia is a broad-spectrum toxicant and is expected to affect all types of fish. It exerts a direct biochemical oxygen demand on the receiving water since dissolved oxygen is consumed as ammonia is oxidized. Moderate depressions of dissolved oxygen are associated with reduced species diversity, while more severe depressions can produce fish kills. Additionally, ammonia can lead to eutrophication, or nutrient over-enrichment, of surface waters. While nutrients are necessary for a healthy ecosystem, the overabundance of nutrients (particularly nitrogen and phosphorus) can lead to nuisance algal blooms.

Toxicology

In freshwater, ammonia has been identified as the major cause of toxicity, owing to the fact that biological membranes are more permeable to it than to the ammonium ion. The membranes of marine organisms, on the other hand, may be more permeable to the ammonium ion. The proportions of ammonia and ammonium are related to pH, temperature, salinity and the permeability of biological membranes to ammonia and ammonium forms. For freshwater, at pH 8.0 and 20°C, the proportion of NH_3 is 3.82%. At pH 9 and 10, the proportions increase to 28.4% and 79.9%, respectively (Eddy 2005). A combined lime treatment and ammonium sulphate application takes advantage of the fact that ammonia is much more toxic to aquatic organisms at higher pH values (Clearwater, Hickey et al. 2008).

Environmental fate and decomposition

Ammonia is a non-persistent and non-cumulative toxicant to aquatic biota, but its decomposition will probably be slow. It might be possible to reduce the post-treatment ammonia toxicity by adding acid to the treatment pond (Clearwater, Hickey et al. 2008).

Formulations and application methods

Urea is a slow-release source of ammonia. When combined with bleaching powder (a source of hypochlorite ions) it yields a long acting biocide resulting from the reactive blend of the two ingredients. Ammonia and hypochlorite ions react to form a number of products, depending on the temperature, their concentrations, and how they are mixed. The main reaction is chlorination of ammonia, first giving chloramine (NH_2Cl), then NHCl_2 and finally nitrogen trichloride (NCl_3). These materials are very irritating to eyes and lungs and are toxic to aquatic organisms above certain concentrations (Clearwater, Hickey et al. 2008). The formation of high levels of chloramines is undesirable owing to its toxicity, persistence and bioaccumulation potential. Therefore, application of the two compounds is not recommend for the treatment of natural ecosystems.

Uses against unwanted fish

In India, researchers applied 12 to 18 ppm of anhydrous ammonia in fish ponds to kill submersed weeds and observed massive fish kill. Prawns and frogs in the treatment area were also killed (Ramachandran and Ramaprabhu 1976). A series of applications of 13 to 40 ppm of anhydrous ammonia in Texas lakes resulted in decimation of local plankton and profound changes in water chemistry, but no persistent residues (Champ, J.T. et al. 1973). Several reports on the harmful effects of NH_3 on fish and aquatic invertebrates have been published, including acute toxicity bioassays in which rainbow trout (*Salmo gairdneri*) and on cutthroat trout (*S. clarki*) were negatively affected by short-term cyclic fluctuations of ammonia (Thurston, Chakoumakos et al. 1981), and studies of the impacts of ammonia on plankton populations (Schlüter and Groeneweg 1985; Arauzo and Valladolid 2003).

Ammonium compounds have also been used to control echinoderms, crustaceans, mollusks (See Chapter III) and microalgae (see Chapter IV). Injections of various poisons including ammonia were found to be locally effective on the Great Barrier Reef to kill the crown-of-thorns starfish *Acanthaster planci* (Birkeland and Lucas 1990; Zhao, Guo et al. 1998). Ammonia was successful agent also against early-stage and late-stage larvae and juveniles of the Chinese mitten crab (*Eriocheir sinensis*) reared

in laboratory. Juveniles were more sensitive than larvae (Zhao, Guo et al. 1998)

Chlorination

(See Chapters III and IV for more information on chlorine compounds and derivatives)

General information

Chlorine-based chemicals that rely on the biocidal action of hypochlorous acid (HClO), hypochlorite ion (ClO⁻) and chlorine dioxide (ClO₂) are commonly used to reduce the level of bacteria, viruses, algae and fungi in domestic water supplies, swimming pools, sewage effluents and industrial water systems (Bolch and Hallegraeff 1993).

These compounds have also been used in aquaculture to kill unwanted fish, larvae and pathogens in culture ponds and tanks. Hypochlorous acid is a non-specific poison that kills most aquatic organisms, including fish (Westers 2001). Chlorine dioxide is also a non-specific biocide, commonly used for disinfection of drinking water. It has been tested for the treatment of potential AIS in ballast water tanks (Gregg, Rigby et al. 2009).

Hypochlorous acid (HClO) is a weak acid that forms when chlorine ions (Cl⁻) react with water (H₂O). This reaction may be initiated by the addition of chlorine gas (Cl₂) or bleach (i.e. calcium hypochlorite, also known as solid bleach or sodium hypochlorite, also known as liquid bleach) into water. Hypochlorous acid further decomposes into hydrogen (H⁺) and hypochlorite ions (ClO⁻). Hypochlorite ions also have biocidal properties albeit much less effective than those of hypochlorous acid. The combined concentration of hypochlorous and hypochlorite ions is referred to as free chlorine or chlorine residual. The dissociation of hypochlorous acid into hydrogen and hypochlorite ions is a pH dependant reaction. At pH 7.5 the concentration of hypochlorous acid and hypochlorite is expected to be about the same.

Chlorine dioxide, on the other hand, dissolves in water but does not react with water (i.e. it does not hydrolyze). Instead, it remains as a dissolved gas in solution. Chlorine dioxide is less affected by ambient pH, and different from other chlorine compounds it does not react with organic compounds or ammonia, thus it does not form harmful byproducts (see *environmental fate and decomposition section*). However, it is explosive under pressure and difficult to transport, therefore it is usually manufactured on site. Chlorine dioxide is usually produced as a watery solution or gas. It is produced in acidic solutions of sodium chlorite (NaClO₂), or sodium chlorate (NaClO₃). For large installations sodium chlorite, chlorine gas (Cl₂), sodium hydrogen chlorite (NaHClO₂) and sulphuric or hydrogen acid are used for the

production of chlorine dioxide on site with a generator. To produce chlorine dioxide gas, hydrochloric acid (HCl) or chlorine is brought together with sodium chlorite (*See formulations and application methods section for more details on the methods used to promote chlorination*).

Calcium hypochlorite has been used since the mid-1930 for sanitation in fish culture facilities and has been recommended for eradication of unwanted fish and tadpole shrimp (*Triops* spp. and *Lepidurus* spp.) in partly drained fish ponds (Panikkar 1960).

For decades, chlorine has remained as the chemical agent of choice to control a wide variety of fouling organisms (e.g. bacteria, algae, fungi and invertebrates) in closed water systems. Its advantages include cheap and flexible availability (in gaseous, liquid and solid forms), ease of dosage and broad spectrum activity (Rajagopal, Venugopalan et al. 2003). The chemistry of power plant chlorination and chlorine byproducts has been reviewed by Jenner et al. (1996).

The toxic properties of chlorine compounds have also been used to control zebra mussels and related nuisance mollusks (see Chapter III) and in rapid-response action for the eradication of invasive mollusks and seaweeds (see Chapters III and IV).

Chlorine compounds and derivatives are registered by the USEPA as general use disinfectants and their maximum input into natural systems are set in water quality standards promulgated by the USEPA and by state and local government.

Mode of action

The broad-spectrum biocidal activity of chlorine is mainly mediated by hypochlorous acid (HClO), which is formed in aqueous solutions at pH 5-8. The toxicity of chlorine is a function of several factors including chlorine concentration, pH, exposure time, and type and quantity of chlorine compounds formed (Gregg, Rigby et al. 2009 and references therein). Hypochlorous acid and hypochlorite's mode of disinfection action emanates from a suite of properties namely the capacity to inhibit glucose oxidation (Knox, Stumpf et al. 1948), interfere in the cells' ability to regulate their adenylate pool and ATP production (Albrich 1981; Barrette, Hannum et al. 1989), inhibit DNA replication (Rosen, Michel et al. 1998) and cause post-translational modifications (i.e. folding and unfolding) to proteins (Winter, Ilbert et al. 2008).

Fish mortality to chlorine has been attributed to general impairment of gill function (Bass, Jr. et al. 1977) and to oxidation of reduced iron (Fe^{+2}) in the hemoglobin to methemoglobin (Fe^{+3}) resulting in inhibition of respiration.

Chlorine seems to inhibit an enzyme system, methemoglobin reductase, that protects red cells from oxidant damage (Grothe and Eaton 1975). In addition, the simple immersion of fish in a low concentration of chlorine (< 1 ppm) causes an immediate and significant efflux of sodium and gain in weight reflecting an ionic and osmotic imbalance.

The toxicity of chlorine has also been shown to be influenced differentially by cations prevalent in natural waters, such as sodium, potassium, calcium and magnesium (Katz 1979).

In addition, the presence of chlorine ions in ambient water may stimulate behavior responses that can lead to negative impacts to certain organisms. For instance, continuous chlorination using chlorine gas or hypochlorite forces closure of the shells of bivalve mollusks. This action cuts off the supply of oxygen and food enriched waters and prevents the expulsion of carbon dioxide and other waste products (Morton, Au et al. 1976).

The mode of disinfection action of chlorine dioxide is not completely elucidated. While it seems like neither dehydrogenases, DNA, nor proteins are the major site of action of chlorine dioxide on bacterial cells, the inhibition of protein synthesis may play a part in cell mortality (Roller, Olivieri et al. 1980; Knapp and Battisti 2001).

Selectivity

Chlorine bleach and chlorine dioxide are powerful non-selective biocides and will kill most bacteria, fungus, virus, algae and may harm or kill some plants and animals depending on the concentration and exposure time (Knapp and Battisti 2001). At a concentration of 5 ppm, chlorine kills most fish species after as little as 1 h of exposure (Westers 2001).

Toxicology

In general, chlorine compounds are hazardous to handle and even short-term exposure to high doses of certain chlorine compounds may be fatal. Chlorine gas is extremely toxic, and for this reason not recommended for field applications. Sodium hypochlorite (i.e. liquid bleach) and calcium hypochlorite (i.e. solid bleach) are oxidizers that promote combustion and that decompose in the presence of heat, water or contamination, with the release of corrosive chlorine gas. Solutions are corrosive to the skin, eyes and upper respiratory tract, but these risks can be eliminated by the use of protective equipment and clothing, and implementation of safety procedures. Long-term exposure to sub-lethal concentrations of fumes and solutions may result in dermatitis, skin lesions and damage to respiratory passages (USEPA 1999; USEPA 2006; Clearwater, Hickey et al. 2008).

A study that investigated the sensitivity of rainbow trout to acute and chronic toxicity of chlorine compounds showed that chlorine dioxide (ClO_2) is less toxic and chlorite (ClO_2^-) much less toxic than chlorine (2 to 4 orders of magnitude). Observed LC_{50} for 96-hour exposure to chlorine dioxide was 2.2 ppm for larvae and 8.3 ppm for adult fish; 20-day LC_{50} for larvae was 1.6 ppm. Chlorite was found to be 48 to 18 times less acutely toxic to larvae and adult fish, respectively (Svecevicus, Syvokiene et al. 2005).

Environmental fate and decomposition

Chlorine bleach is nonpersistent in water. Chlorine gas reacts with water and forms hydrochloric acid and hypochlorous acid. This last component may break down into hydrogen and hypochlorite ions, depending on ambient pH. Calcium hypochlorite (i.e. solid bleach) hydrolyzes into hypochlorous acid and calcium hydroxide while sodium hypochlorite (i.e. liquid bleach) hydrolyzes into hypochlorous acid and hydroxide (Knapp and Battisti 2001).

The major problem with using these precursors for chlorination is that free chlorine reacts with some organic compounds that are abundant in natural waters forming persistent chlorinated organic by-products (e.g. trihalomethanes, haloacetic acids and chlorite) in low concentrations. These compounds are of concern because they have been shown to be carcinogenic to laboratory animals. Also, if ammonia and other nitrogenous substances are present when chlorine is introduced, chloramines form. These are persistent and toxic to fishes and all other forms of aquatic life (Westers 2001). Neutralization of chlorine can be done with sodium sulphate or sodium thiosulfate, and this is recommended, especially if the treatment area is part of a public water supply system or is ecologically sensitive (Clearwater, Hickey et al. 2008 and references therein).

In contrast, chlorine dioxide does not involve or create free available chlorine or chlorinated byproducts. The major by-products resulting from chlorine dioxide disinfection are chlorite, chlorate, and organic, biodegradable by-products such as carbonyl compounds and short chain carboxylic acids. Chlorate has been documented to be toxic to marine micro-algae, particularly in nitrate limited waters, but only at high concentrations (Gregg, Rigby et al. 2009 and references therein).

Formulations and application methods:

Chlorine can be dosed to water in a variety of forms including liquefied chlorine gas, sodium hypochlorite or calcium hypochlorite. Most frequently, a sodium hypochlorite solution is applied directly into water. Household

chlorine bleach is 3-6% liquid solution of sodium hypochlorite, which can also be found in powder (bleach powder). A 12% solution is widely used in waterworks for the chlorination of water and a 15% solution is more commonly used for disinfection of waste water in treatment plants. High-test hypochlorite (HTH) is sold for chlorination of swimming pools and contains approximately 30% calcium hypochlorite. The crystalline salt is also sold for the same use; this salt usually contains less than 50% of calcium hypochlorite. However, the level of "active chlorine" may be much higher. During the rapid-response action to eradicate *Caulerpa taxifolia* in South California, managers found that using pellets of calcium hypochlorite, rather than injection liquid sodium hypochlorite into the containment units was easier and safer (Anderson 2005).

Chlorine dioxide is rarely transported, because of its explosiveness and instability. It is usually manufactured on site. Chlorine dioxide is usually produced as a watery solution or gas. It is produced in acidic solutions of sodium chlorite (NaClO_2), or sodium chlorate (NaClO_3). For large installations sodium chlorite, chlorine gas (Cl_2), sodium hydrogen chlorite (NaHClO_2) and sulphuric or hydrogen acid are used for the production of chlorine dioxide on site. To produce chlorine dioxide gas, hydrochloric acid (HCl) or chlorine is brought together with sodium chlorite. This is usually done with a generator of some sort. The best way to store chlorine dioxide is as a liquid at 4 °C. At this state it is fairly stable. Chlorine dioxide cannot be stored for too long, because it slowly dissociates into chlorine and oxygen. When concentrations are higher than 10% chlorine dioxide in air, there is an explosion hazard. In a watery solution, chlorine dioxide remain stable and soluble. Watery solutions containing approximately 1% ClO_2 (10 g L^{-1}) can safely be stored, under the condition that they are protected from light and heat interference (USEPA 1999).

Use against unwanted fish

In India, a combination of commercial bleaching powder (5 ppm of chlorine) and urea (5 mg total ammonia) proved effective in killing murrel fry (*Channa punctatus*) under laboratory conditions. When a similar combination was tried under field conditions, the most efficient results were obtained in ponds where urea had been broadcast 24 to 48 h before the application of bleaching powder (Ram, Rao et al. 1988). These authors pointed out that the advantages of the method are ease of operation, quick restoration of normal pond conditions and, above all, reduced costs.

Similarly, urea combined with bleaching powder (at 3 and 5 ppm, respectively) has been found to be the threshold limit for effective fish kill within 1 hr of application under laboratory and field conditions, while crabs

and snails were killed within 24 hrs. Combined residual chlorine appears to be the toxic factor (Mohanty, Chatterjee et al. 1993).

A review of the use of chlorine as a possible fish toxicant reported results from laboratory bioassays in which 96-h LC₅₀ values ranged from 0.17 mg L⁻¹ for rainbow trout (*Salmo gairdneri*) to 1.41 mg L⁻¹ for black bullhead (*Ameiurus melas*)(Ashley 1989).

Chlorine was considered as an extra barrier for the introduction of nonnative species within the Garrison Diversion project (Marking, Bills et al. 1983). This project proposed to transfer Missouri River water to a large part of eastern North Dakota for agricultural and industrial uses. The authors concluded that concentrations >2 mg L⁻¹ of chlorine would effectively eliminate eggs and larvae of common carp and rainbow smelt.

Copper compounds and derivatives

(See Chapters III and IV for more information on copper)

General information

Copper is a naturally-occurring, ubiquitous element in the environment that is found in water, air, and occurs naturally in various foods, bound to macromolecules rather than as a free ion. For many animals, copper is essential for the homeostasis of life.

Copper compounds are registered for use on virtually all food/feed crops, on various ornamental crops, and also for use in a number of aquatic sites and for different purposes, most commonly in algacide and herbicide applications for maintenance of aquaculture facilities, drainage systems, ponds, fountains, lakes, reservoirs, sewage lagoons, stocking and irrigation canals and sewer system.

Copper pesticides are formulated using various copper compounds, which ultimately dissociate into cupric ion, the actual toxic agent. Copper hydroxide (Cu(OH)₂) is a broad-spectrum fungicide and bactericide for terrestrial use, while copper sulfate (CuSO₄·5H₂O) is the active ingredient of registered aquatic herbicides, algacides and molluscicides. Copper sulfate is approved for use in ponds, lakes and reservoirs for different purposes, including the control freshwater snails that may be a vector for harmful trematodes, as well as to control leeches, and tadpole shrimp (*Triops* spp. and *Lepidurus* spp..) in rice fields (USEPA 2006; USEPA 2008).

The application of copper sulfate for the purpose of killing entire fish populations dates back to at least the early 1900's. The use of the compound as a fish toxicant continued, but complete kills of fish are not

always obtained, and frequent side effects include decimation of non-target phytoplankton, zooplankton, insect larvae, and mollusks. These deficiencies, plus the associated risks and the advent of rotenone and other fish toxicants, caused the use of copper sulfate in fish control to decline (Lennon, Hunn et al. 1971).

While copper is not specifically approved for the control of unwanted fish, it could be used for this purpose if applied in accordance with the restrictions specified in the end-use product's label.

Water Quality Standards for the State of Hawaii (HAR 11-54) establishes maximum copper concentration in all waters to be 6 µg per liter of freshwater and 2.9 µg per liter of saltwater.

Mode of action

Copper, though an essential heavy metal for living organisms, has proven to be toxic in excessive amount. Essential metals are only required by living beings in trace amounts and the excess concentration is regulated by homeostatic control mechanisms. If the supply concentration is too high, the homeostatic mechanism ceases to function and the intake of metal will impose acute or chronic effects to the organism (Peña and Pocsidio 2007).

The main cause of copper toxicity to fish and aquatic invertebrates is through rapid binding of copper to the gill membranes, which causes damage and interferes with osmoregulatory processes. The amount of cupric ion in the environment, and its toxicity to aquatic animals through gill damage, is dependent on a number of water quality parameters including pH, alkalinity, and dissolved organic carbon (USEPA 2006; USEPA 2008).

Selectivity

Copper sulfate is very toxic to fish and to other groups of aquatic organisms including mollusks. Even at low rates of application, this material may be poisonous to trout and other species. Its toxicity to fish generally decreases as water hardness increases. Fish eggs are more resistant than young fish fry to the toxic effects of copper sulfate. Direct application of copper sulfate to water may cause significant decrease in populations of aquatic invertebrates, plants and fish (EXTOXNET 1995).

Toxicology

Copper sulfate is only moderately toxic to humans and other mammals upon acute oral exposure, but it is corrosive to the skin and eyes. It is readily absorbed through the skin and can produce a burning pain, along with the same severe symptoms of poisoning from ingestion. The biochemical processes that underlie the chronic effects of copper sulfate are not well understood by science.

Long-term effects are more likely in individuals with Wilson's disease, a condition which causes excessive absorption and storage of copper and chronic exposure to low levels of copper can lead to anemia. Copper sulfate poses less of a threat to birds than to other animals. It is toxic to several potential non-target aquatic invertebrates, such as crab, shrimp and oysters. Bees are endangered by strong, water-based copper compounds. Most animal life in soil, including large earthworms are eliminated by the extensive use of copper-containing fungicides in orchards. High concentrations of copper are toxic to aquatic organisms and may cause a significant decrease in populations of aquatic invertebrates, plants, and fish (EXTOXNET 1995; USEPA 2006).

One of the limiting factors in the use of copper compounds is their serious potential for phytotoxicity, or poisonous activity in plants. Copper sulfate can kill plants by disrupting photosynthesis.

In addition, Dafforn et al. (2008) found that copper enhanced early recruitment of several non-indigenous species, whereas it reduced recruitment of indigenous species.

Environmental fate and decomposition

Copper is strongly adsorbed to clay and organic matter, and remains in soil indefinitely. It is partly washed down to lower soil levels by water percolating through the ground; partly bound to soil components; and partly changed into different metabolites, or breakdown products. Copper is considered to be among the more mobile of the heavy metals in surface environments. Although copper sulfate is highly water soluble, the copper ions are strongly adsorbed or precipitated to soil particles when it is applied to soil. Copper is bound, or adsorbed, to organic materials, and to clay and mineral surfaces. The degree of copper adsorption to soils depends on the level of acidity or alkalinity of the soil. The distance that it can travel in soil is limited by its strong adsorption to many types of surfaces. All applied copper will become a part of the soil copper content. The leaching potential of this material is low in all but sandy soils. No evidence has been found to show that this material gets removed from water through volatilization (EXTOXNET 1995; USEPA 2006).

Due to its high water solubility, excessive amounts of copper sulfate should be kept out of lakes, streams and ponds. High concentrations of copper are toxic to aquatic organisms and may cause a significant decrease in populations of aquatic invertebrates, plants, and fish (EXTOXNET 1995).

Copper bioaccumulates in the food web, and is highly persistent in the environment. It is stored primarily in the liver, brain, heart, kidney and muscles (EXTOXNET 1995; USEPA 2006).

Formulations and application methods

Copper sulfate can be found in the market under the names bluestone, blue vitriol, Salzburg vitriol, Roman vitriol, and blue copper.

Use against unwanted fish

There are reports that bluegill reproduction in an Ohio farm pond was controlled by dropping crystals of copper sulfate into nests containing eggs or fry, but other authors had little or no success with the same technique in eight lakes in Michigan. The compound was reported as successful in the selective control of shad (*Alosa* spp.), suckers (Catostomidae family), and bullheads in 10 lakes in Texas where total alkalinity was under 100 ppm. The State of Virginia made great use of copper sulfate in fishery management in the 1960's. The toxicant used to be applied to ponds of 0.4 to 60.7 ha for the selective control of rough fish (Lennon, Hunn et al. 1971).

Lime compounds and derivatives

(See Chapters III and IV for more information on lime compounds and derivatives)

General information

Lime is a general term for calcium-containing inorganic materials, in which carbonates, oxides and hydroxides predominate. The term lime usually refers to calcium oxide (i.e. CaO, also called burn lime or quicklime) or calcium hydroxide (i.e. Ca(OH)₂, also called slacked lime or hydrated lime).

Both, quicklime and hydrated lime are commonly used in aquaculture operations to manipulate water and soil chemistry and increase productivity in earthen ponds. Liming with limestone is used to increase the pH of acidic soils and enhance organic decomposition. Increased alkalinity will stabilize the pH of pond water, which can vary from pH 6 to pH 10 during the day if the alkalinity is less than 20 mg CaCO₃. L⁻¹ (Wurts and Masser 2004)

At high application rates, burnt and hydrated lime can be used to disinfect ponds by temporarily increasing soil and water pH and alkalinity to levels sufficient to kill any parasites and micro-organisms present (Piper, McElwain et al. 1982).

Hydrated lime is sometimes added to anaerobic marine environments for odor control, to inhibit sulfate-reducing bacteria, and to precipitate algae, silt and phosphorous to the bottom (Nishimura and Seki 1983; Muezzinoğlu et al. 2000). In odor control treatments of marine systems, 114 g m^{-2} (i.e. $1.14 \text{ tonnes ha}^{-1}$) of hydrated lime killed 99% of the sulfate-reducing bacteria and 66% of anaerobic bacteria, and 198 g m^{-2} ($1.98 \text{ tonnes ha}^{-1}$) killed 99.8% of the anaerobic bacteria (Muezzinoğlu et al. 2000). These are short-term improvements which persist only as long as additions of lime are made.

Liming can also be used to eradicate aquatic pest species by applying lime compounds at exceedingly high rates. In order to be effective against the majority of aquatic species the pH must be increased to 12 for at least 24 h. The technique may be ineffective against organisms that can avoid or limit their exposure by strategies such as burrowing (Piper, McElwain et al. 1982).

Quicklime has been used in Prince Edward Island estuaries and in Long Island Sound, Canada, as a treatment to clear oyster beds of starfish (Needler 1940; MacKenzie 1977) and other fouling organisms. In California to eliminate sea urchins from kelp beds (Bernstein and Welsford 1982) (see more information about the use of lime for fouling control in Chapter III).

Locke et al (2009) suggested that the addition of hydrated lime could have two positive consequences at the ecosystem level: countering the acidification of ocean waters, and improving water quality in eutrophic systems.

Mode of action

The most likely mechanism of liming toxicity is that extreme changes in water chemistry cause direct physical damage to the gills of most aquatic organisms and disrupt respiration and iono-regulation. The majority of aquatic animals would probably be killed by caustic injury to the gills and other delicate respiratory and dermal surfaces. Macrophytes will also be physically damaged by extreme alkalinity (Piper, McElwain et al. 1982; Clearwater, Hickey et al. 2008).

Selectivity

Although there are a few reports of successful fish control projects that applied liming as the main treatment method (Lennon, Hunn et al. 1971; Clearwater, Hickey et al. 2008), it seems like most adult fish are resistant to the toxic effects of lime (Locke, Doe et al. 2009 and references therein).

Toxicology

Burnt lime is a strongly caustic agent which may cause severe irritation of the skin and mucous membranes (Budavari et al. 1989). Hydrated lime has usually been used in field-scale aquatic operations in order to minimize human health and safety concerns. Hydrated lime can irritate the skin and mucous membranes but is significantly less caustic and reactive than burnt lime.

Hydrated lime is considerably less toxic than quicklime (usually only 15-20% as many mortalities when exposed for the same duration), but hydrated lime may have a longer exposure time (North and Shaefer 1963).

Macroalgae (*Macrocystis pyrifera*, *Gigartina canaliculata*, *G. leptorhynchos*, and *Egregia laevigata* Setchell); adults and larvae of blue mussel, eastern oyster, quahog *Mercenaria mercenaria*, softshell clam *Mya arenaria*; adult and juvenile (carapace length 37 mm) American lobster *Homarus americanus*; adult spiny lobster *Panulirus interruptus*; shrimps; some polychaetes; gastropods with opercula; encrusting corals; anemones; some sponges; and most fish species have survived heavy applications (5- 50 tonnes ha⁻¹) of quicklime (Galtsoff and Loosanoff 1939; Needler 1940; Loosanoff and Engle 1942; North and Shaefer 1963; McKenzie 1977; Shumway et al. 1988). Some of these organisms survived exposure to quicklime in tanks for more than one year. Histological examination after six months of exposure revealed no damage to American lobster gills, mussel gills or worm parapodia, and gross examination showed no damage to any tested species other than starfish (Shumway et al. 1988). Marine organisms that are unaffected by quicklime are not expected to be affected by hydrated lime, as it is a decomposition product of quicklime in sea water.

Similarly, organisms affected by quicklime may be susceptible to hydrated lime. At heavy application rates, quicklime was lethal to all echinoderms (i.e. starfish, urchins, sea cucumbers); it also harmed jellyfish, some sponges, mature gastropods lacking opercula (e.g., abalone *Haliotis* sp.), larval gastropods, some polychaetes, bryozoans, larval American lobsters, some larval fishes, and adult flatfishes (Loosanoff and Engle 1942; Shumway et al. 1988 and references). Sublethal effects on the filtering and growth of eastern oysters and softshelled clams were observed in laboratory experiments (Loosanoff and Engle 1942; Turner 1970). Eggs and fry of winter flounder, *Pseudopleuronectes americanus*, survived in strong

solutions of quicklime, filtered to remove particles, but not in solutions where they contacted solid particles (Loosanoff and Engle 1942).

Loosanoff and Engle (1942) found that direct contact with particles of quicklime was lethal to stage 1, 2 and 3 American lobster larvae. Stage 1 larvae were the most resistant and survived only 40 min in solutions with quicklime particles. In filtered 9% solution, larvae survived 4 h (Loosanoff and Engle 1942). As a result of this series of experiments, Loosanoff and Engle (1942) and Shumway et al. (1988) recommended that quicklime not be used when larvae of lobsters and flatfish were present in the water.

Much of the published literature on lime effects in the marine environment relates to quicklime and less is known about hydrated lime. The mechanism of toxicity of both chemicals is closely associated with their strongly alkaline nature (pH~ 12 in saturated solution). For example, similar lesions were caused to the aboral surface of starfish by quicklime settling through the water column, and to the oral surface by crawling over a deposited layer of hydrated lime (Loosanoff and Engle 1942).

Environmental fate and decomposition

Quicklime rapidly converts to hydrated lime after mixing with water (<15 min.), in an exothermic reaction which liberates extreme heat (152.8 kcal mole⁻¹) and may burn the surface of susceptible organisms on contact (North and Shaefer 1963, Bernstein and Welsford 1982).

The subsequent conversion of hydrated lime to calcium carbonate is slower, liberates less heat and requires carbon dioxide. The duration of this conversion is an important determinant of the overall effect of hydrated lime. The rate of conversion from hydrated lime to calcium carbonate depends on temperature, the particle size of the lime, and the availability of carbon dioxide (typically present at 0.009 kg m⁻³ of sea water, and required at a rate of 0.59 kg of carbon dioxide to convert 1 kg of hydrated lime) as well as related ions such as bicarbonate which affect the equilibrium state (North and Shaefer 1963).

Complete conversion of heavy applications (>5 tonnes ha⁻¹) of quicklime to calcium carbonate requires five to 18 days in sea water and most of this time is used in the conversion of hydrated lime to calcium carbonate (North and Shaefer 1963; McKenzie 1977; Bernstein and Welsford 1982). Using pH as an indicator of lime conversion, Loosanoff and Engle (1942) found that lighter applications of quicklime (~0.9 tonne ha⁻¹) increased pH by only 0.2- 0.4 pH units to 8.2- 8.5, and conversion to calcium carbonate occurred within two days at 2 °C.

Limed ponds treated at moderate application rates for aquaculture conditioning or disinfection can be restocked approximately 10 days after treatment or when the pH has decreased to below 9.5 (normal pH = 6–8). Pond water productivity (e.g. phyto- and zooplankton levels) will not return to normal until 3 or 4 weeks after treatment. Recovery of pre-treatment conditions usually takes longer if the pH has been maintained above 12 for several days (Clearwater, Hickey et al. 2008).

Only limited follow-up field information on the recovery of hardness and alkalinity to pre-treatment concentrations was available. Extremely high alkalinity is likely to be more detrimental to aquatic organisms than extremely high hardness. Reduction of alkalinity to concentrations acceptable for aquatic plants and invertebrates following liming may take some considerable time. Australasian field data show that pre-treatment conditions were achieved within 24 h in some cases. In others, pH was still elevated after 3 weeks and 3 to 5 months post-treatment, despite the application of acid to assist recovery (Clearwater, Hickey et al. 2008).

The use of lowest possible application rates and strategies such as addition of ammonium sulphate can help to ensure rapid recovery to pre-treatment conditions. Liming of eutrophic waters can cause precipitation of phosphorus and this precipitate will remain on the sediment after treatment (Wang et al. 2005). Debates over the persistence of lime in the environment are probably due to different formulations used. Lime can dissolve in about 48 hours leaving no residue. However, in field trials, lime remained effective for several weeks after deposition, but at a much reduced kill rate (McEnulty, Bax et al. 2001).

Formulations and application methods

The efficacy of treatment is significantly dependent upon the solubility of the lime and smaller particle sizes will dissolve more readily (Boyd and Masuda 1994). Burnt lime and hydrated lime are much more soluble in water than limestone and therefore are preferred in liming operations when pond sterilization and pest eradication are the goals and rapid change in pH and alkalinity is required (Boyd & Tucker 1998).

Lime can be found in crystals or powder formulations. It can be applied to the dried pond bottom or the water surface. When used to eradicate pest aquatic organisms it is added as hydrated lime to water at high applications rates sufficient to increase pH > 2 for 4 to 5 days. More moderate applications of lime are used to manipulate pond water and soil chemistry (i.e. alkalinity, hardness and pH) to enhance aquaculture operations (Boyd and Masuda 1994; Boyd and Tucker 1998; Wedemeyer 2001).

The typical procedure for a liming operation is to decrease the water volume to a manageable size (and filtering the extracted water to remove pest organisms including their eggs and larvae), then to remove as many of the remaining aquatic organisms as possible by netting and trapping. Lime is then applied (preferably as a pre-mixed slurry) to the remaining water to rapidly increase the pH to 12. This high pH is maintained for 4–5 days, or another compound such as ammonium sulphate is added to rapidly kill any resistant organisms. The exposed banks and vegetation can also be treated with lime if necessary (Clearwater, Hickey et al. 2008).

Piper et al. (1982) suggested that an application rate equivalent to 0.1– 0.3 kg m⁻³ is required to raise the pH above 10. Evidence from field work for pest eradication in Australia and New Zealand suggests that rates of 0.6– 3.1 kg/m³ are required to maintain the pH sufficiently high to eradicate pests (i.e. pH > 12 for 4–5 days). One operation used 44.4 kg m⁻³ to eradicate koi carp and maintain pH at 11.1 for 0.5 h. Exact application rates will depend on the local soil and water chemistry (specifically, the pH and alkalinity) and the quality of the (usually hydrated) lime (Boyd and Tucker 1998).

Quicklime is more unstable than hydrated lime and will absorb CO₂ and water from air; therefore, it must be stored in dry, air-tight conditions.

Uses against unwanted fish

Liming has been used in eradication attempts against various fish species, including *Gambusia* spp., goldfish (*Carrasius auratus*), grass carp (*Ctenopharyngodon idella*), gudgeon (*Gobio gobio*), koi carp (*C. carpio*), rudd (*Scardinius erythrophthalmus*), tench (*Tinca tinca*) and against freshwater crayfishes, including marron (*Cherax tenuimanus*), and yabbies (*Cherax destructor*). Limited follow-up data indicate that the eradication of pest fishes was generally successful, and that crustaceans (marron and yabbies) were resistant when pH was < 12 (Clearwater, Hickey et al. 2008). The application of lime has also been reported as a control method for predator fish in prawn-culture ponds and in carp nursery ponds in China (Lennon, Hunn et al. 1971) but details about these control efforts are not available.

Neem

General information

Neem is natural substance derived from the oil found in the seeds of the neem tree (*Azadirachta indica*). Humans have used this naturally-occurring

oil for millennia for medicinal, cosmetic, and pesticidal purposes. Neem has not been traditionally used as a piscicide but recent efforts have attempted to develop stable neem oil emulsions that could be used for the control unwanted species, including fish.

Two active ingredients with herbicidal properties are derived from the oil found in the neem seeds: azadirachtin and clarified hydrophobic extract of neem oil. When used in pesticide products, both azadirachtin and clarified hydrophobic extract of neem oil can be applied to many food and non-food crops indoors and outdoors to control certain insects and related pests. Labels direct users not to contaminate water and not to apply when honeybees are actively visiting flowers in the area (USEPA 2001).

Mode of action

Not clear. Research has shown that the presence of neem leaves' extract in water interfered with the antioxidant defense system of the neotropical fish *Prochilodus*. Fish exposed to various neem extract concentrations exhibited damaged gill and kidney tissue, and the authors concluded that although neem extract is less toxic to *P. lineatus* than other synthetic insecticides used in fish-farming (e.g. carbamates and organophosphates such as carbofuran and malathion) it does cause functional and morphological changes in this fish species (Winkaler, Santos et al. 2007).

Selectivity

Neem is reported as extremely toxic to all aquatic life, but no specific information regarding selectivity among fish species could be found.

Toxicology

Neem is not expected be harmful to humans nor to other mammals. For centuries, traditional Indian medicine has used neem preparations for various therapeutic purposes, some of which required the ingestion or dermal application of neem products by patients. While some cases of fatal acute poisoning incidents exist, these seem to be few and usually involving overdosing through oral intake (Jacobson and Schmutterer 2005).

Acute lethal effects of two neem-based formulations on eight species of macroinvertebrates were determined in flow-through screening tests at 10 times the expected environmental concentration of 0.35 mg L⁻¹. Significant mortality occurred only in the may fly *Isonychia bicolor/rufa* exposed to one formulation, and further tests were conducted to determine a concentration-dependent response. The LC₅₀ for *I. bicolor/rufa* was estimated at 1.12 mg

L⁻¹. Three detritivorous species were tested at expected environmental concentrations at longer exposures in aquatic microcosms to determine effects of the two formulations on feeding rates and survival. Neither formulation caused significant mortality or antifeedant effects after a 28-day exposure (Kreutzweiser 1997).

Results from an *in situ* microcosms study showed that the use of a neem-based insecticide (Margosan-O) in or near aquatic environments could lead to disturbances in benthic populations and may cause decreases in numbers of organisms that are important in food web and nutrient cycling processes (Scott and Kaushik 2000).

Another study evaluated short-term acute toxicity of two neem-based insecticides (Neemix™ and Bioneem™) to six aquatic animals: crayfish (*Procambarus clarkii*), white shrimp (*Penaeus setiferus*), grass shrimp (*Palaemonetes pugio*), blue crabs (*Callinectes sapidus*), water fleas (*Daphnia pulex*), and mosquito larvae (*Culex quinquefasciatus*). The risk was calculated using the level of concern endpoints (Q values) and relative hazard index (RHI) for acute and chronic exposure scenarios. The Q values of Neemix™ and Bioneem™ derived from acute exposure tests indicated that *D. pulex* was the only sensitive species to the test. RHI values of Neemix™ and Bioneem™ for *D. pulex* were above the critical limit of 10 indicating that these pesticides may pose a moderate hazard to this species and related crustaceans in acute exposure scenarios. The RHI values of the two pesticides were all below the critical limit of 10 for *P. clarkii*, *P. setiferus*, *P. pugio*, *C. sapidus*, and *C. quinquefasciatus*. This aquatic risk assessment process showed that the risk values of tested pesticides did not exceed the criteria, and the authors concluded that no ecological hazard is likely to result from their use (Goktepe, Portier et al. 2005).

Formulations and application methods

Experiments with natural surfactants like castor-oil, nonylphenol, sodium lauryl sulphate, and calcium alkyl benzene sulphonate have been carried out in order to develop stable emulsions. Apparently, methanol can be used to prepare neem extract based emulsifiable concentrates. Larvasidal effects of emulsions and emulsifiable concentrates have been examined and laboratory experiments have shown effectiveness of these emulsions and emulsifiable concentrates as pesticide (Waghmare, Ware et al. 2007).

Saponins

(See Chapter III for more information on saponins)

General information

The term “saponin” is used to refer to a wide variety of naturally occurring water-soluble glycosides that can be extracted from hundreds of plants species as well as in some bacteria and in certain lower marine animals such as sea cucumbers and starfishes (Riguera 1997; Yoshiki, Kudou et al. 1998; Kaushik 2005).

Saponins derive their name from their ability to form stable, soap-like foams in aqueous solutions.

Several sources of saponins have been used for centuries by indigenous tribes of various parts of the world as narcotics to capture fish for eating purposes. For instance, the utilization of *Barringtonia asiatica* (hutu or hotu in the Hawaiian language), a mangrove tree known for its active saponin content (Herlt, Mander et al. 2002) as a fishing tool by native peoples of Samoa and Hawaii is well documented in the literature (Stokes 1921; Cox 1979; Bishop, Baker et al. 1982).

Tea-seed cake is probably the most common saponin-source used in the control of nuisance fishes around the world these days. Tea-seed cake powder is a byproduct of the extraction of tea seed oil from *Camellia* sp.. It is commonly applied for the control of unwanted fish, especially in penaeid shrimp farms in Asia (Tang 1961; Chen, Chen et al. 1996; Chen and Chen 1997).

According to Lennon et al. (1971), Russian researchers evaluated various sources of saponins with piscicidal properties and confirmed the toxicity of glycosides extracted from azalea flowers to fish and other aquatic life, but stated that the chief source of saponins used for killing unwanted fish from inland waters in the region was sugar beet. Saponins occur mostly in the surface layer, rootlets, and tail of the beet. Concentrates of saponins are obtained by centrifuging the foam on beet-pressing water, resulting from the pressing of beets into briquettes. The liquid concentrate is toxic to fish at 0.2 ppm whereas the dry saponins are toxic at 2 ppm. The toxic action on fish occurs within 20 to 24 hours, and decomposition of the saponins is complete within 7 to 10 days. The authors attest that the saponins from sugar beets are the most effective and acceptable means for ridding inland waters of nuisance fishes.

Mode of action

Saponins have haemolytic properties (i.e. capacity to break open red blood cells, causing the release of hemoglobin into the surrounding fluid), certain fungal, bacterial and viral growth inhibitory properties, and have a

pronounced toxic action against mollusks and organisms which use gills for breathing, such as amphibians and fish. The exact mode of this toxic action, however, is still obscure (Hostettmann and Martson 1995; Francis, Kerem et al. 2002).

Selectivity

Not clear, but expected to be low among fish species. Chiayvareesajja et al. (1997) described the use of tea-seed cake as a piscicide in earthen ponds at a concentration of 25 ppm against five fishes: walking catfish, common carp, mosquitofish, tilapia, and silver barb. Mortalities of the five species ranged from 28% to 65% after 24 hrs of exposure. They found that the ponds could be restocked 4 days after applying the piscicide.

Toxicology

Toxicological assessments for saponins are scarce. The toxicity of saponins to humans, as well as to non-target species varies greatly depending on the type of saponin in question, specifically on its molecular structure. In general, very little is known about the enzymes and biochemical pathways involved in saponin biosynthesis. The genetic machinery required for the production of this type of vegetal secondary metabolite is as yet largely uncharacterized, despite the considerable commercial interest in this important group of natural products. This is due in part to the complexity of the molecules and the lack of commercially available pathway intermediates for biochemical studies (Francis, Kerem et al. 2002).

While assessments of tea seed cake powder toxicity are limited, two references point out the negative impacts that tea seed cake powder saponins exert on oxygen uptake, ammonia-N and urea excretion, hemolymph oxyhemocyanin, protein levels, acid-base balance, and ammonia excretions by one species of saltwater shrimp (*Penaeus japonicus*) exposed to saponins at different salinity levels (Chen and Chen 1996; Chen and Chen 1997).

Formulations and application methods

Of all saponins, quinoa-extracted saponins (i.e. triterpene bidesmosidic glycosides of oleanolic acid, hederagenin, and phytolaccagenic acid) are the only ones known to be active compounds of a pesticide registered in the U.S., under the trade name Heads Up[®] Plant Protectant (USEPA 2005). The product consists of a seedling protectant. Preliminary studies of its

toxicity to goldfish and tilapia indicate that quinoa-extracted saponins do not possess piscicidal properties (San Martín, Ndjoko et al. 2008).

Use against unwanted fish

The use of tea-seed cake for control of undesirable fish in ponds before stocking is customary for Chinese and Taiwanese fish farmers (Tang 1961). A review of fish toxicants (Lennon, Hunn et al. 1971) cites publications that report the eradication of fish with tea-seed cake from shrimp ponds in Malaya, China and Singapore since at least the early 1900's. The use of tea-seed cake is also an ancient practice to kill all unwanted species including eels, mullets, seabasses and tilapia from aquaculture ponds prior to stocking for shrimp farming in India. Following their decomposition and decrease in toxicity, the piscicides act as organic fertilizers (Clearwater, Hickey et al. 2008).

Chiayvareesajja et al. (1997) described the use of tea-seed cake as a piscicide in earthen ponds at a concentration of 25 ppm against five fishes: walking catfish, common carp, mosquitofish, tilapia, and silver barb. Mortalities of the five species ranged from 28% to 65% after 24 hrs of exposure. They found that the ponds could be restocked 4 days after applying the piscicide.

Tea-seed cake has also been used as a poison against fishes at a concentration of 15 ppm, and it may also be used as a fertilizer to condition ponds prior to stocking with shrimp, and to stimulate moulting in shrimp (Sarkhel and Das 2005).

In the United States, experiments with low concentrations of tea-seed cake saponins in a small shrimp pond in South Carolina killed most fish species (not specified) but, at the time, the authors concluded that such treatment would be cost prohibitive in this country (Lennon 1970).

Fish Anesthetics (TMS and Clove oil derivatives)

Anesthetics are chemical or physical agents that calm animals and cause them to progressively lose their mobility, equilibrium, consciousness, and finally their reflex action. Most anesthetics can produce several levels or stages of anesthesia., ranging from sedation, common anesthesia, surgical anesthesia and death. The stage achieved usually depends on the dose and the length of exposure. When an anesthetic is first administered fish may become hyperactive for a few seconds (Coyle, Durborow et al. 2004).

Anesthesia, euthanasia, and sedation of wild and captive fish are common requirements in aquaculture, fisheries research, and management around the world. Although still uncommon, the use of anesthetics to facilitate capture and removal of invasive fish species is a useful AIS control tool, especially in circumstances in which the application of piscicides is precluded.

Anesthetics that have been used in aquaculture include chemical methods, such as Tricaine methanesulfonate (i.e. TMS or MS-222), Benzocaine, Quinaldine, 2-Phenoxyethanol, Metomidate, Carbon dioxide, and clove oil derivatives (e.g. eugenol and isogenol) as well as non-chemical methods, such as hypothermia and electro-anesthesia. These have been reviewed by Coyle et al (2004).

MS-222 is the only anesthetic currently licensed in the US. It is commonly used for anesthesia, sedation, or euthanasia of fishes (mainly salmonids). TMS is a muscle relaxant that operates by blocking action potentials which stops signal exchange between the brain and the extremities. MS-222 is easily soluble in fresh and salt water and drastically decreases water pH, increasing ambient acidity, which may be toxic for fish. Baking soda (i.e. sodium bicarbonate) is commonly used to buffer the solution to a pH range of 6.5-7.5. Usually twice the amount of buffer is added to attain the neutral pH. Buffering may not be necessary in marine water, because sea water itself has buffering capacity. When used in fish production, MS-222 requires a 21-day withdrawal after treatment and before human consumption (Holloway, Keene et al. 2004; King, Hooper et al. 2005).

The active ingredient in AQUI-S™ is food-grade isoeugenol (about 12% cis-isomer and 88% trans-isomer). AQUI-S™ is a fish anesthetic and sedative approved for use in several countries including Australia, Chile, and New Zealand and is a candidate for U.S. approval as an anesthetic and sedative with a reduced withdrawal time.

AQUI-S is 50% isoeugenol (i.e. 540 g isoeugenol L⁻¹) plus an excipient (polysorbate) that improves emulsification of the compound with water. The excipient is a biodegradable, food-grade compound. AQUI-S is not the same as clove oil. Clove oil is 80–85% eugenol, with 15–20% impurities, some of which are toxic (Clearwater, Hickey et al. 2008 and references therein).

Few studies have been undertaken to assess the effect of eugenol, isoeugenol or AQUI-S on aquatic plants and invertebrates.

Acute toxicity and anesthetic effects of clove oil (i.e. 80% eugenol dissolved in ethanol; final concentration of 800 mg L⁻¹) were studied in the estuarine/marine Green tiger prawn (*Penaeus semisulcatus*). The 1-h EC₅₀ and LC₅₀ and the 24-h LC₅₀ were calculated at concentrations of 25, 130 and 30 mg L⁻¹, respectively, at 30 °C, salinity 40 ppt, pH 8.6 and dissolved oxygen > 6 mg L⁻¹. Generally, with increasing concentrations of clove oil, the times required for sedation and anesthesia decreased, while the recovery times increased. According to the LC₅₀ calculation obtained in this study, clove oil can be classified as a low toxic substance for *P. semisulcatus* weighing

1.8–2.1 g. Most prawns exposed to high concentrations of clove oil required resuscitation, and the risk of ventilatory failure increased with increasing dose of clove oil. This is probably because of the physical properties of clove oil, where it coats anatomic structures (Soltani, Marmari et al. 2004).

An industry-generated human and environmental risk assessment of isoeugenol for use as a fragrance in products such as washing powders, cleaning sprays and detergents, reported that no toxicological data were available for algae or fish. In a daphnid immobilization test, the EC_0 , EC_{50} and EC_{100} were 3.8, 7.5 and 15 $mg L^{-1}$, respectively, after 48 h of exposure. In an unpublished study on the toxicity of isoeugenol conducted in New Zealand, the 72-h EC_{50} for a freshwater algae (*Pseudokirchneriella subcapitata*) was 10.4 $mg L^{-1}$, and the NOEC8 was 4.8 $mg L^{-1}$. Juvenile rainbow trout (*Oncorhynchus mykiss*) were more sensitive than the algae with 96-h LC_{50} of 5.1 $mg L^{-1}$ and NOEC of 4.2 $mg L^{-1}$. The 48-h cladoceran (*Daphnia magna*) immobility was less sensitive than either algal growth or fish survival, with 24-h EC_{50} of 17.9 $mg L^{-1}$ and NOEC of 6.3 $mg L^{-1}$. The 21-day chronic *D. magna* reproduction test yielded the most sensitive endpoint with EC_{50} of 1.1 $mg L^{-1}$ and NOEC of 0.4 $mg L^{-1}$ (Clearwater, Hickey et al. 2008 and references therein).

AQUI-S™ New Zealand Ltd. is currently pursuing approval for AQUI-S™ in the United States to sedate market-sized salmonids during harvest (“rested harvest” claim) and to fully anesthetize fish for aquaculture management procedures (“general anesthesia” claim). Legal use of AQUI-S™ as an anesthetic in U.S. fish culture depends on approval by the FDA. Attributes of a drug that must be characterized before the drug is approved include (1) characterizing the depletion of a drug's total residues after exposure and (2) characterizing the depletion of a marker residue (a marker residue is the parent compound, a metabolite, or combination of residues that persist for the longest time in the target tissue, e.g. edible fillet tissue).

In a Guidance for Industry document issued in 2007, the FDA restated that neither clove oil nor any of its components are the subject of an approved new animal drug application and, because of safety concerns, should not be used as an anesthetic in fish. In that document, the FDA explained that results from tests conducted under the National Toxicology Program that indicate eugenol is an equivocal carcinogen and methyleugenol is carcinogenic to rodents, and that because some clove oil products may contain or include either methyleugenol or isoeugenol, or both, FDA is concerned that the use of clove oil or its components in fish may adversely affect human food safety and animal food safety. This concern especially applies to the use of clove oil or any of its components in fish intended for use in human or animal food, and from use in those fish that may be released into public waters where they would be available as food for other aquatic species, or could be caught and end up in the human food supply. In addition, because clove oil and its components have not been evaluated for target animal safety, the FDA is also concerned that the use of any of these compounds may adversely affect fish, including endangered aquatic species (FDA 2007).

Comparative Analysis

Since prehistoric times, cultures throughout the world have used piscicidal plants for killing fish (Eldredge 1987). In recent times, scientists have identified many of the plant compounds responsible for killing the fish and have found that these compounds possess other important biological properties, such as insecticidal and anti-cancer activities (Cannon, Burton et al. 2004).

Various plant extracts have a long history of traditional use as fish poison in the Pacific region as well. Some of these plants have their chemical nature well explained by science such as the rotenone-source “auhuhu”, (*Tephrosia purpurea*) and the saponin-source “hutu” or “futu” (*Barringtonia asiatica*). Others, however, have not had their mode of action completely elicited. Species of the genus *Wikstroemia*, for instance, have long been known for their piscicidal effects (Stokes 1921; Cox 1979; Bishop, Baker et al. 1982), but few studies (Gupta and Gillett 1969) have attempted to characterize its chemotoxic properties. Unfortunately, insufficient research on potential long-term effects of these poisons to non-target species currently precludes their safe utilization for the control of AIS. Also, plant extracts can only be applied for pesticidal purposes if they are either registered by the USEPA for these types of use or classified by that agency as exempted under 25(b) of the FIFRA (for more information, see the *Pesticide Laws Section* in Chapter I).

Rotenone and antimycin A are the only two pesticides currently registered for fish control activities in the US. While these two toxicants are analogous in many aspects, such as in their similar mode of action, they do have certain characteristics that make each of them more suitable to some environments and to some target species than to others. For instance, in large bodies of standing water, antimycin A is not as effective as rotenone primarily because antimycin A decomposes too quickly and is difficult to disperse throughout the water column. Antimycin A tends to give better results in running water, streams, and shallow waters while rotenone can be efficient in either lotic or lentic environments (Turner, Jacobson et al. 2007).

On the other hand, when compared to rotenone, antimycin A is often regarded as being less harmful to non-target aquatic organisms (especially to invertebrates). Results from a study that examined impacts of antimycin and a combination of antimycin and rotenone on benthic invertebrates in several high elevation

streams in Bridger-Teton National Forest (Wyoming) indicate that antimycin had little to no effect on aquatic macroinvertebrates, while rotenone additions dramatically increased invertebrate drift and caused significant mortality in bioassays (Cerreto, Jr. et al. 2003; Cerreto, Hall et al. 2004). Nevertheless, another study of immediate and short-term effects of antimycin A on macroinvertebrates during a fish renovation project in Fossil Creek, Arizona indicated that there is a high end concentration at which antimycin A can have significant deleterious effects on aquatic invertebrates (Dinger and Marks 2007). These authors found that at the highest dose used (100 µg/L), drift was five times the pretreatment drift level and invertebrate standing stocks in pools and riffles decreased immediately. Densities rebounded in riffles within 5 months but remained depressed in pools. At the lower concentration (.54 µg/L), macroinvertebrate mortality, measured as increased drift, was 24 times the pretreatment level. At this lower concentration, however, macroinvertebrate densities in the benthos were not reduced. Under both concentrations, species composition shifted toward more tolerant species. Although antimycin A effects were mostly short term, several species were locally extirpated. The authors found no explanation for the loss of some species over others.

Lennon et al. (1971) emphasized that antimycin is more selective than rotenone, is effective at low concentrations in a wide range of water quality, is not repulsive to fish and leaves no toxic residue. Furthermore, rotenone adsorbs quickly to suspended particles and bottom sediments (Gilderhus 1982; Dawson, Gingerrich et al. 1991) and this may reduce treatment efficiency in turbid waters, a situation in which the use of antimycin A may be a valid alternative. Nevertheless, many questions remain regarding antimycin A's toxicity, metabolism and fate in the environment (USEPA 2006).

USEPA's alternative analysis determined that the cost of an antimycin A treatment, based upon 1998 data, was comparable to that of rotenone. While antimycin A may be more costly per unit volume of product, it requires lower application rates than rotenone (USEPA 2006).

There are gaps in what concerns the understanding of potential long-term impacts of the application of either rotenone or antimycin A to aquatic systems. However, when contrasting the number of studies that have been published regarding the toxicology and environmental fate of these compounds, it is obvious that rotenone's chemistry and effects have been much more studied than antimycin A's chemistry and effects. That is

only logical, considering that rotenone has been used as fish toxicant and insecticide for centuries in various parts of the world, while the isolation of antimycin A is a fairly recent development. Furthermore, while gaps in knowledge remain, the level of uncertainty regarding the known facts about rotenone seems to be lower than regarding known facts about antimycin A.

Finally, the number of registered end-use products and companies that manufacture rotenone-based products is substantially greater than the corresponding for antimycin A. In fact, there is currently only one company (Aquabiotics Ltd.) that produces a single antimycin A-based toxicant (Fintrol). Ultimately, this discrepancy in the variety of products and registrants represents a limitation for managers when choosing among formulations, application methods and non-active ingredients that best suit their eradication and control projects. For these reasons, our understanding is that when planning projects where either toxicant is suitable, managers should favor rotenone. The use of antimycin A should be reserved for situations in which the use of rotenone will clearly yield inferior results, such as in applications in ecosystems where one cannot risk the survival of aquatic invertebrates.

The development of more selective piscicides that could serve as alternatives to rotenone and antimycin A would be especially valuable to reduce impacts on native species and perhaps on endangered fauna and flora. As it can be observed in Table 1, most substances known for their piscicidal properties are either low in selectivity or not selective at all, and many will require complete kill not only of fish but of all fauna that comes in contact with the toxin. Among the non-registered piscicides presented in this chapter, saponins seem to be the most promising options. Like rotenone and antimycin A, these are non-synthetic vegetal extracts with desirable piscicidal properties and may be suitable to situations in which the use of either rotenone or antimycin A is precluded.

Chlorine has been used for fish control but its application for this type of use is not recommended, especially in natural systems. The main problem with using chlorine compounds for the control of unwanted species are potential direct harmful effects to non-target species and, in the cases of chlorination with gas or bleach, the formation of ecotoxic trihalomethanes (THMs) and chloramines by reaction with organic material in the water (Clearwater, Hickey et al. 2008; Gregg, Rigby et al. 2009 and

references therein). That and the amount of chlorine necessary to kill unwanted fish are expected to make the project unfeasible. Copper is also a poor option for fish control, as it would be required in large quantities and because copper is persistent in the environment and bioaccumulates in the food web, this type of use in open systems would represent significant environmental risk.

Liming and ammonia may also kill fish, but because using these active ingredients for these purposes has not been systematically studied, much remains unknown about the effect of factors such as application rate, the exposure time required to kill pest species, pest species resistance, and recovery rates of treated water bodies. More toxicological and decomposition studies for all groups of biopesticides are necessary, and their current regulatory status restrict their use for fish control purposes. Neem and saponins seem to be promising alternative methods for fish control as these are organic in nature. However, little is known about their actual efficacy ranges, biodegradation processes, and byproducts. More studies are needed to fully assess these products' acute and chronic toxicity to target and non-target aquatic species.

In conclusion, due to the urgent need to control invasive nuisance fish in Hawaiian waters, especially in fresh and brackish environments, I recommend that experiments are conducted to 1) further the understanding dispersion and degradation rates for rotenone and antimycin A when applied to specific ecosystems of interests (e.g. anchialine pools; streams) in variable but characteristic ranges of environmental conditions (e.g. various water and air temperatures; different wind conditions); 2) resolve the uncertainties that remain regarding antimycin A's effects on non-target aquatic species, decomposition processes, and degradation rates; 3) explore new fronts in phytochemistry and molecular biology in order to identify alternative piscicides, and advance our knowledge about promising compounds such as saponins.

Table 1. Comparative Table for Piscicides

	Selectivity	Known sensitive fish	Known tolerant fish	Decomposition	Leaching potential	Volatility	Byproducts and their toxicity	Risk to non-target aquatic organisms	Risk to birds and terrestrial spp.	Efficacy restraints
Antimycin A	Intermediate (at 5-10ppm) to Low (at 10-20ppb)	Salmons, trouts, Minnows	At low conc.: Ictalurids (Bullhead, catfish) and invertebrates	Within hours to a month	Low	Low	Blastmycic acid, antimycin lactone and antimycic acid (others?) Low toxicity	Intermediate (at 5-10 ppm)	Apparently low	pH
Rotenone	Intermediate (at 50-150 ppb) to Low (at 150-200 ppb)	Salmon, trouts, sunfish, bass, pike, carps	At low conc.: Bullheads, monnow, catfish	Within hours to weeks (faster in warmer, clear waters with pH>9)	Low, except in sandy soils	Low	Rotelanone. Low toxicity	High (50-200 ppb)	Low	T, light/ turbidity, suspended organic matter, pH
Ammonia	Low	Unknown	Unknown	Slow (?)	Low-intermediate	Low-intermediate	If in contact with hypochlorite ions it forms chloramines which are very toxic	High	Unknown	pH, T, S, exposure time
Liming	Low	Carps, mosquito fish, rudd, tench, gudgeon	Unknown	Months for reestablishment of original pH/ alkalinity	Unknown	Low???	None	Intermediate to High	Low???	Solubility of the liming compound

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Chlorine	Low	Trout, carp, smelt	Black bullhead	Weeks	Low	Intermediate-High?	Reacts with N and forms chloramines: highly toxic and persistent; reacts with organic matter and forms trihalometanes: carcinogenic	High	Intermediate	Ph
Copper	Low	Bullheads, suckers, shad	Unknown	Highly persistent	Low except in sandy soils	Low	Copper ion: heavy metal, bioaccumulative	High	High due to bioaccumulation	pH, organic matter content
Saponins	Intermediate-Low	Eels, mullets, sea bass, tilapia, catfish, carp, mosquitofish	Unknown	Unknown	Unknown	Low	Unknown	Intermediate-High	Low???	Unknown

Chapter III: Aquatic Molluscicides/ Anti-fouling Pesticides

Various species of mollusks have been identified as non-native and potential nuisance in freshwater (Englund, Arakaki et al. 2000; Yamamoto and Tagawa 2000) and marine (Eldredge and Smith 2001) environments of Hawaii. Some of these species have caused substantial negative impacts to human health, the environment and local economies. The giant African snail (*Achastina fulica*), for instance, carries a parasite (rat lungworm) that causes serious disease (eosinophilic meningoencephalitis) in humans, besides posing a threat to native plants and competing with native species for resources (Staples and Cowie 2001). The golden apple snail (*Pomacea canaliculata*) is a major pest in taro fields, eating the stem, corms and other plant parts and causing substantial losses for taro producers (Levin 2006).

The list of established non-native fouling organisms in Hawaii includes the freshwater Asiatic clams (*Corbicula fluminea*), marine clams (e.g. *Anomia nobilis*, *Crassostrea virginica*, *Chama macerophylla*), bryozoans (e.g. *Schizoporella* cf. *errata*, *Bugula neritina*), ascidians (e.g. *Ascidia sydneiensis*, *Didemnum candidum*), polychaetes (e.g. *Chaetopterus* sp., *Sabellastarte spectabilis*), and crustaceans (e.g. *Balanus amphitrite*, *Chthamalus proteus*) (Eldredge & Smith 2001). Ecological and economic impacts of these invasions in Hawaii remain largely unquantified but their negative impacts are for the most part conspicuous.

Also conspicuous is the constant possibility of new introductions, mainly through our limited capacity to manage hull fouling. Hull fouling has been identified as the probable vector of introduction of more than 70% of all established non-indigenous species in coastal North America (Fofonoff, Ruiz et al. 2003), and is believed to be responsible for more successful marine introductions than any other mechanism in Hawaii (Eldredge and Carlton 2002). Measures to prevent AIS introductions via hull fouling include husbandry practices, such as periodic dry-docking for hull maintenance, and the application of biocidal anti-fouling paints and coatings to vessel hulls.

Biocidal antifouling coatings deter the attachment of fouling organisms by leaching toxic compounds, such as those that contain tributyltin (TBT), copper, and zinc. Because these compounds are also detrimental to non-target organisms, many regions have adopted or are considering restrictions on their use. Tributyltin (TBT) is a highly effective antifouling agent that has been restricted by many nations in line with the 2001 International Maritime Organization (IMO) Convention on the Control of Antifouling Systems on Ships (Takata, Falkner et al. 2006).

A review of antifouling coatings falls outside the scope of this report but can be found elsewhere (Yebra, Kiil et al. 2004; Coutts and Forrest 2007; Hellio and Yebra 2009). Instead, here we focus on evaluating chemical ingredients that could be used for rapid-response efforts (e.g. hull sterilization for grounded vessels) and post-introduction control programs.

Next, I review active ingredients that are currently registered by the USEPA to be applied in aquatic ecosystems for mollusk control and then present various products that have been identified as potential molluscicides for aquatic systems. At the end of the chapter, I provide a comparative analysis of the selected active ingredients, scrutinize their advantages and disadvantages and highlight the major data deficiencies related to the topic.

USEPA-registered Aquatic Molluscicides

Copper compounds and derivatives

(See Chapters II and IV for more information on copper)

Mode of action

Copper derivatives apparently affect slugs and snails through contact with the surface epithelium (Thompson, Sibley et al. 2005). Copper sulfate (up to 2500 ppm Cu) was relatively ineffective when injected into the hemolymph of the aquatic snail *Biomphalaria glabrata* but had a toxic effect when exposed to the snail's surface epithelium (Sullivan 1976).

Uses against unwanted mollusks and other fouling animals

Copper's pesticidal efficacy has been tested against various mollusks and fouling organisms including common barnacles, polychaetes, bryozoans, starfish and tunicates.

A recent study evaluated the influence of copper on feeding rate, growth, and reproduction of the golden apple snail *Pomacea canaliculata*. Ten days of exposure to a relatively high concentration of copper ($67.5 \mu\text{g L}^{-1}$) reduced snails' feeding rate and retarded their growth. Exposure to $20 \mu\text{g L}^{-1}$ after 36 days increased feeding rate to 28%. After 20 days of exposure at $30 \mu\text{g L}^{-1}$, snail growth was significant but thereafter declined. Copper did not affect reproduction (Peña and Pocsidio 2007).

Copper sulfate has been experimentally used in Hawaii as a molluscicide to control *Pomacea canaliculata* infestations in taro patches. The program was conducted in the early 1990's by the HDOA, but discontinued mainly due to concerns regarding potential deleterious effects to the environment and unanswered questions regarding cupric toxicity for taro itself. While research indicated that copper content in taro leaf blades had not increased with copper sulfate treatment, it did increase in taro roots, and various concurrent morphological alterations were observed in the plants growing in treated patches (Hofstra and Clayton; Hill and Miyasaka 2000; Levin 2006).

Results from a study that compared toxicity and selectivity of 18 chemicals to two sizes of zebra mussels (*Dreissena polymorpha*), two nontarget fish (rainbow trout, *Oncorhynchus mykiss* and channel catfish, *Ictalurus punctatus*), and an unionid mussel (threehorn wartyback *Obliquaria reflexa*) indicate that copper sulfate was toxic to zebra mussel (LC_{50} values ranging from 1 to 10 mgL^{-1}) but inferior to potassium chloride, Bayluscide, and Clamtrol CT (i.e. ammonium compound) as a control option when considering its low selectivity (Waller, Rach et al. 1993). Nevertheless, McMahon and Tsou (1990) found that copper was lethal to zebra mussels, with 5 ppm copper ions for 24 hr giving 100 percent kill of veligers.

The survival and behavior of specimens of blue mussel *Mytilus edulis* exposed to discontinuous and continuous copper regimes was investigated in both fluctuating salinity conditions and in constant full strength sea water. Continued addition of 0.5 and 0.25 ppm of copper caused damage to mussels within 1-2 days. In full strength sea water a 6 h off 0.5 ppm Cu Super(2+) regime caused no damage at all in 5 days and it was found that this was because *Mytilus* can detect copper in its environment and close its shell valves to avoid the detrimental consequences of exposure to copper. In fluctuating salinity regimes it was found that the timing of copper delivery was extremely important; animals survived copper delivery occurring at low or falling salinities because of interacting closure responses to copper and low salinities (Davenport 1977). Another study with blue mussel *Mytilus edulis*, found that the effects of copper on embryo development were

different between mussel populations depending on the level of pollution of the site of these population's origin (Hoare, Beaumont et al. 1995).

The black striped mussel *Mytilopsis sallei* seems to be more resistant to copper than *Crassostrea gigas* and *C. virginica*) and blue mussel *M. edulis* with LC50 values of 0.6 mg L⁻¹ for 4 days, 1.9 mg L⁻¹ for 4 days, 0.103 mg L⁻¹ for 2 days (embryos) and 0.141.0 mg L⁻¹ for 2-7 days respectively (McEnulty, Bax et al. 2001).

Historically, the eradication of *Mytilopsis sallei* from Darwin Harbor, Australia in 1999 is one of the few examples where an established marine invader has been eradicated from a site. The marinas were closed off from the surrounding waters by gates, quarantined and chemically treated with sodium hypochlorite (i.e. liquid bleach) and copper sulphate. Specifically, 0.5 tonne of copper sulphate was dissolved and added to the waters of Tipperary Waters Estate Marina, producing a total concentration of 1.5 mg L⁻¹ of copper in the ambient water. Diving surveys conducted 3 days after the application confirmed that all *Mytilopsis* sp. had been killed. No other chemical was used at this site besides copper sulphate (Bax, Hayes et al. 2002).

Copper is toxic for freshwater clams (*Corbicula fluminea*), especially to juveniles. Longer-term exposure of these clams to copper at 8.4–26.7 µg Cu L⁻¹ in artificial streams resulted in impairment of growth. In studies conducted at the Clinch River, Virginia power plant, clam growth was reduced at 22.5–104.8 µg L⁻¹ at a water hardness (180 mg L⁻¹) that was more than two times the hardness in artificial streams (Belanger, Farris et al. 1990).

A solution containing 0.0006mg Cu/mL killed the barnacles *Balanus balanoides* and *Balanus eburneus* in two days (Kerkut and Munday 1962).

A study that reviewed available information on the sensitivity of marine and estuarine organisms to copper reported the results of 14-day exposures of *Boccardia proboscidea* to clean sediment spiked with a series of copper concentrations. The 14 day LC₅₀ values ranged from 303 to >384 µg Cu per gram and results for growth were also variable (McPherson and Chapman 2000).

The toxicological impact on hard fouling species caused by the presence of anti-marine-borer timber preservative chromated copper (Arsenate) in timber exposed in the marine environment was minimal to the invasive tunicates *Ascidella aspersa* and *Ciona intestinalis* (and also to *Elminius modestus*, *Hydroides ezoensis*, and *Electra pilosa*) dominating the surface of test panels after 6 months exposure (Brown and Eaton 2001).

The attachment of the bryozoan larvae (*Bugula neritina*) to vertical copper, mercury and control paint strips in slowly moving sea water was compared by Wisely (1962). The number of organisms attached to the control strips was seven times greater than to the copper strip and twenty times greater than to the mercury strip.

The attachment of bryozoans *Bugula neritina* was also impaired by copper paint surfaces by repelling or killing the larvae and by inhibiting growth and metamorphosis of attached larvae. Growth of *Bugula* ancestrulae in sea-water solutions of copper is inversely proportional to the concentration up to 0.3 mg. per liter. Higher concentrations completely inhibit growth (Miller 1946).

A more recent study tested the tolerance of four introduced bryozoans (*Bugula neritina*, *Watersipora subtorquata*, *Schizoporella errata* and *Tricellaria occidentalis*) to a range of copper concentrations (0, 10, 50, 100 and 500 $\mu\text{g l}^{-1}$). Recruits of all species survived in 0 and 10 $\mu\text{g l}^{-1}$ Cu for 20 days, with only *Bugula neritina* and *Watersipora subtorquata* recruits surviving exposure to 50 and 100 $\mu\text{g l}^{-1}$ Cu. *B. neritina* and *W. subtorquata* colonies exhibited reduced post-metamorphic growth in 50 $\mu\text{g l}^{-1}$ Cu compared to controls, with no growth observed in 100 $\mu\text{g l}^{-1}$ Cu. Growth for *S. errata* and *T. occidentalis* was higher at 0 $\mu\text{g l}^{-1}$ than 10 $\mu\text{g l}^{-1}$ copper. Post-exposure growth of surviving colonies was assessed by transplanting colonies to the field. *W. subtorquata* colonies exposed to 50 $\mu\text{g l}^{-1}$ Cu were the only colonies showed decreased survival and growth post copper-exposure. Overall, *B. neritina* and *W. subtorquata* showed the greatest tolerance to copper (Piola and Johnston 2006).

Tunicate immune reactions are profoundly affected by exposure to TBT and copper. The antigenic structure of haemocytes of *Styela plicata* was substantially affected by TBT and copper (Radford, Hutchinson et al. 2000).

The use of copper sulfate through microencapsulation showed limited success against the parasitic sabellid worms (*Terebrasabella heterouncinata*) in abalone culture facilities (Shields, Buchal et al. 1998).

Injection of poisons using pole spears was found to be locally effective on the Great Barrier Reef to control crown-of-thorns starfish *Acanthaster planci*. Kill rates were close to 100% with copper sulphate recommended as the safest and easiest to use at a maximum rate of 140 injections per hour. Due to the non-specificity of copper the oysters were also affected (Birkeland and Lucas 1990).

Niclosamide

General information

Niclosamide is a relatively selective, non-cumulative chlorinated aromatic amide pesticide. It is used as a molluscicide to control freshwater snails which carry the vectors for diseases which affect fish and humans and, in tributaries to the Great Lakes, the Finger Lakes and Lake Champlain, as a lampricide to control sea lamprey larvae. Less than 400 pounds of active ingredient niclosamide is used each year in lamprey and freshwater snail treatments. Niclosamide has been used as a human and veterinary drug for treatment of parasites (USEPA 1999).

Mode of action

The mode of action of niclosamide is not completely elucidated. The available data suggests that it can act mostly on respiration and carbohydrate metabolism and that the nitro group is fundamental for the activity. The reduced niclosamide, 2,5'-dichloro-4'-aminosalicylanilide, has been shown to lose all molluscicidal and cestocidal properties, being also ineffective in decoupling respiration and electron transport linked phosphorylation. Also, some evidence of the interaction of niclosamide and DNA, suggests that niclosamide toxicity can be caused by this interaction, after reductive activation (Abreu, Goulart et al. 2002).

Selectivity

At concentrations normally used to kill slugs for human parasites control, niclosamide is expected to be of low selectivity to mollusks and amphibians, but relatively higher selectivity to other groups of non-target species, including fish (Andrews, Thyssen et al. 1982; Perrett and Whitfield 1996; Oliveira-Filho and Paumgarten 2000). However, Oliveira-Filho & Paumgarten (2000) found that niclosamide killed various non-target species, including planktonic crustacea (*Artemia* sp.), fishes (*Danio rerio*, *Poecilia reticulata*) and frog tadpoles (*Rana catesbeiana*) at concentrations much lower than that which killed *Pomacea* sp..

Toxicology

Niclosamide is classified by the World Health Organization (WHO) and by the USEPA as a pesticide of very low toxicity to mammals (acute oral LD₅₀ values of >1000 ppm; acute dermal toxicity LD₅₀ >2000 ppm). There is no evidence that niclosamide causes developmental toxicity, mutagenicity or

carcinogenicity. It can be moderately toxic to aquatic vertebrates (e.g. fishes and amphibians), crustacean and is extremely toxic to mollusks (USEPA 1999; WHO 2003).

Niclosamide is the parent compound of the commercial product Bayluscide®. Bayluscide® was found to be less toxic to striped bass (*Morone saxatilis*) fingerlings (, $LC_{50} = 1.05 \text{ mgL}^{-1}$ for 72 h exposure) than to freshwater snails. It is considered as toxic to crayfish, frogs and other aquatic organisms but at concentrations higher than those normally used to kill snails (WHO 2003). However, Oliveira-Filho & Paumgarten (2000) found that niclosamide killed various non-target species, including planktonic crustacea (*Artemia* sp.), fishes (*Danio rerio*, *Poecilia reticulata*) and frog tadpoles (*Rana catesbeiana*) at concentrations much lower than that which killed *Pomacea* sp.. These authors registered a LC_{50} for *Pomacea* sp. of 1.29 (0.64-2.86) mgL^{-1} for 24 h exposure, while the LC_{50} for all other organisms was under 0.70 mgL^{-1} (lowest LC_{50} of 0.28 mgL^{-1} for *D. rerio*, and highest of 0.60 mgL^{-1} for *P. reticulata*).

Exposure of a catfish species, (*Clarias lazera*), to sublethal concentrations of Bayluscide® (0.1 and 0.3 ppm) for a 6-month period induced 14% and 95% mortalities, respectively. In addition, there was a depletion of hemopoietic tissue in the mesonephros and spleen, an increased susceptibility to pathogens and appearance of nodules containing infectious organisms. Surviving fish produced lower titers of antibodies against injected antigens than controls and the number of corpuscles of Stannius increased in proportion to the concentration and duration of exposure to the Bayluscide. Long term exposure probably induced immunosuppression in *C. lazera* which caused these changes (Faisal, Cooper et al. 1988).

There may be adverse affects on tadpoles (Eastern spade foot toad, *Scaphiopus holbrooki*, to North American bullfrog *Rana catesbeiana*; LC_{50} of 0.198 mg L^{-1}), and mosquitofish (*Gambusia holbrooki*; LC_{50} of 0.657 mg L^{-1}) at high application rates used to eliminate freshwater snails ($LC_{50} = 0.062\text{--}0.085 \text{ mg L}^{-1}$ and $LC_{99} = 0.149\text{--}0.440 \text{ mg L}^{-1}$). At higher concentrations, turtles would be adversely affected. A LC_{50} of 4.909 mgL^{-1} was observed for the red-eared slider (*Trachemys scripta*) (Francis-Floyd, Gildea et al. 1997).

No adverse effects were noted in non-target crayfishes *Procambarus alleni* and *P. paeninsulanus* (10 mg L^{-1} for 24 h).

Three freshwater algae (*Scenedesmus dimorphus*, *S. quadricauda* and *Ankistrodesmus falcatus*) were found to be as sensitivity to Bayluscide® as freshwater snails, with 48-h EC_{50} values of 0.270, 0.175 and 0.176 mg L^{-1} , respectively (Ibrahim 1987).

The marine unicellular alga *Skeletonema costatum* showed an EC_{50} of 0.064– 0.081 mg L⁻¹ (Ibrahim 1983) and growth of the marine alga *Chondrus crispus* was sensitive to 24 h exposures of niclosamide at concentrations ranging from 0.1 to 0.5 mg L⁻¹ (Staples, Shacklock et al. 1995).

Water quality alters the toxicity of Bayluscide. For example, high temperature, low pH, low hardness and low salinity seem to increase the toxicity of the compound to snails (Tchounwou, Englande et al. 1992). At pH < 7, however, the solubility of niclosamide decreases (Nettles, Staats et al. 2001).

Environmental fate and decomposition

Evidence indicates that niclosamide is non-persistent in the aquatic environment. After an application of 1.1 kg ha⁻¹ to a 0.06-ha pond, niclosamide was detected in the water at a concentration of 0.2– 0.4 mg L⁻¹ after 1 h, and at 0.2 mg L⁻¹ after 25 h. In the pond sediment, niclosamide was transiently detected 7 h after treatment, but not 18 h or 25 h after treatment (i.e. < 0.3 mg L⁻¹) (Francis-Floyd, Gildea et al. 1997)

Niclosamide is removed from natural waters by photochemical decomposition, biodegradation and binding to sediments. Although niclosamide is taken up by organisms, bioconcentration factors are considered not significant and it appears to be rapidly metabolized and depurated (Nettles, Staats et al. 2001). In most aquatic environments, niclosamide will adsorb to suspended solids and sediment but this binding is reversible. It is unclear what role, if any, aerobic and anaerobic microbial degradation plays in the dissipation of niclosamide in the aquatic environment (USEPA 1999).

Formulations and application methods

Slow-release and 'bottom-release' formulations are available to target different species (Dawson, Bills et al. 1998). Slow-release formulations in ethylene-vinylacetate copolymer (EVA) are available for the long-term control of snails (El-Nagar, Pfister et al. 1991).

Uses against unwanted mollusks and other fouling animals

Niclosamide was lethal to the aquatic snails *Melanoides turberculatus*, *Physella hendersoni*, and *Planorbella duryi* with LC_{50} = 0.062– 0.085 mg L⁻¹ and LC_{99} = 0.149–0.440 mg L⁻¹ (Francis-Floyd et al. 1997). Niclosamide was also used against two other freshwater snail species (*Helisoma trivolvis*

and *Biomphalaria havanensis*) resulting in 100% mortality after 24 h of exposure to 2 mg L⁻¹ (Tchounwou, Englande et al. 1991).

Infestations of zebra mussel (*Dreissena polymorpha*) in water intakes and pipes in Europe and in the US have been controlled using niclosamide (Waller, Rach et al. 1993). These authors compared Baylucide to other 17 chemical products, including quaternary ammonium compounds, antimycin and potassium permanganate and found that Baylucide was the most toxic chemical to zebra mussels. They also registered that niclosamide is quite selective; it was 2.2 to 2.8 times more selective for zebra mussels than for the next most sensitive organism tested (i.e. channel catfish).

The freshwater oligochaete *Dero digitata* was targeted in catfish rearing ponds in the US and the 24-h LC₅₀ observed was of 0.24 mg L⁻¹ (Mischke and Terhune 2001).

In Brazil, a Baylucide® concentration of 0.30 mg L⁻¹ was 100% effective in the control of the freshwater snails *Biomphalaria stramineus* and *Amularia* spp, but did not harm four species of tilapia (*Tilapia hornorum*, *T. rendalli* and the hybrid *T. hornorum* × *T. niloticum*). Concentrations of 0.45– 0.55 mg L⁻¹ had different effects on the different tilapia species, and 0.75 mg L⁻¹ was lethal for all of the fish species (Rezende de Melo and Studart Gurgel 1981).

Selected Non-registered Aquatic Molluscicides/ Anti-fouling Pesticides

Acetic Acid (Vinegar)

(See Chapter IV, pages 142-143, for more information on acetic acid)

General information

Acetic acid, also known as ethanoic acid, is a common organic acid found in all living organisms. Pure, water-free acetic acid (i.e. glacial acetic acid) is a colorless hygroscopic liquid that freezes at 16.7 °C (62 °F) to a colorless crystalline solid. Vinegar is the diluted form of acetic acid and consists of approximately 5% acetic acid and 95% water.

Acetic acid is a weak acid, in that it is only partially dissociated in aqueous solution. It is often the predominant low molecular weight organic acid produced by microbial fermentation associated with the decomposition of organic matter under anaerobic conditions in aquatic sediments. Acetic acid concentrations in sediments vary with habitat, season, and depth (Spencer and Ksander 1995).

Acetic acid is registered in many countries for use as an organic herbicide in terrestrial applications. In the US, acetic acid is used as a preservative for post harvest stored grains and hay intended for livestock feed. It is also applied as a non-selective herbicide for control of broadleaf weeds and weed grasses. Pesticide products containing acetic acid are used to control non-cropland areas such as railroad rights-of way, golf courses, open space, driveways, industrial sites. Acetic acid is not registered for aquatic applications in waters of the US (USEPA 2008).

Acetic acid has been used to control biofouling in aquaculture areas and associated structures in Canada (LeBlanc, Davidson et al. 2007; Locke, Doe et al. 2009) and in New Zealand (Coutts and Forrest 2005; Forrest, Hopkins et al. 2007; Denny 2008), and has been tested as a treatment method to control biofouling attached to the hulls of vessels and harbor structures (Pannell and Coutts 2007).

In addition, acetic acid has been tested for the control of biofouling algal species in aquaculture settings (Sharp, MacNair et al. 2006; Forrest, Hopkins et al. 2007) and is a known inhibitor of sprouting and growth of freshwater vascular plants, as well as the salt marsh common reed *Phragmites australis* (Spencer and Ksander 1995; Spencer and Ksander 1997; Armstrong and Armstrong 2001). More information about the use of acetic acid for the control of aquatic plants and algae can be found in Chapter IV.

Mode of action

The activity of dissolved hydrogen ions determines the acidity or basicity of a solution and is measured by that solution's potential of Hydrogen (i.e. pH, or the logarithm of the reciprocal of hydrogen-ion concentration in gram atoms per liter). The pH of ambient water is a key factor affecting the rates of many important biological processes like photosynthesis and other metabolic processes. Changes in ambient pH will affect organisms differently, causing various forms of physiological stress. Acetic acid has the ability to alter pH. However, evidence that acetic acid causes toxicity at various low and high pH values (Lynch 1977) and that acetic acid is a more effective biocide than other acids adjusted to the same pH, indicates that the toxicity of acetic acid is not caused by effects of the hydrogen ion alone. In other words, the toxicity of acetic acid is primarily a function of the compound itself rather than altered pH (Forrest, Hopkins et al. 2007; Breidt Jr., Hayes et al. 2004). One possible associated toxic agent is the acetate ion. At pH < 6.5, sodium acetate acts to uncouple cellular communication by disrupting the gap junction channels between cells (Germain and Ancil 1996; Locke, Doe et al. 2009). For instance, in plants, acetic acid causes

leakiness of cell membranes, loss of ions from roots, and occlusions of cell membranes, resulting in reduced permeability to oxygen, accompanied by reduced uptake of water and nutrients (Spencer and Ksander 1995; Spencer and Ksander 1997).

Selectivity

At concentrations $\geq 4\%$, acetic acid is expected to be somewhat poisonous to most aquatic organisms, especially to soft-bodied sessile taxa.

4% acetic acid is lethal to blue mussels, bryozoans and polychaetes at exposures between 1 and 4 minutes (Forrest, Hopkins et al. 2007). However, bivalves are usually able to perceive changes in water chemistry and may avoid the effects of acetic acid by closing their valves. According to LeBlanc et al. (2007), if the intention is to spare the bivalves, the mussels can be shaken to provoke valve closure so that their soft tissue is not exposed to acetic acid. Mortality and loss of attachment can be minimized by 5- 20% with this technique.

Toxicology

At concentrations typically used to control fouling species (4- 8%), acetic acid is food grade vinegar, thus it is not expected to be harmful to human health or to other mammals in general. Applicators and mixers should use equipment to protect against eye and skin irritation that all acids can cause (USEPA 2008).

Locke et al. (2009) evaluated the toxicity of 5% acetic acid to an AIS, the vase tunicate *Ciona intestinalis* and to representatives of non-target taxa in Prince Edward Island (Canada) namely, a luminescent bacteria (*Vibrio fischeri*), a crustacean (sand shrimp, *Crangon septemspinosa*), and a species of fish (threespine stickleback, *Gasterosteus aculeatus*).

Acute toxicity bioassays registered no mortality for threespine stickleback immersed in 5% acetic acid at concentration $\leq 100 \text{ mg L}^{-1}$, but 100% mortality was observed at $\geq 320 \text{ mg L}^{-1}$ (96-h LC_{50} was 178 mg L^{-1} at pH 5.0). Acute toxicity test for the sand shrimp resulted in no mortality at 5% acetic acid concentrations $\leq 50 \text{ mg L}^{-1}$ but 100% mortality was observed at $\geq 500 \text{ mg L}^{-1}$ (96-h LC_{50} was 158 mg L^{-1} at pH 5.5). In chronic toxicity tests (14 days) with sand shrimp, 5% acetic acid did not affect shrimp growth, but 15% mortality was observed at concentration $\leq 100 \text{ mg L}^{-1}$ and all shrimp died at $\geq 320 \text{ mg L}^{-1}$ (96-h LC_{50} was 116 mg L^{-1} at pH 7.0). IC_{50} for bacteria was 88.5 mg L^{-1} (pH 6.3).

Some fish (e.g. sand smelt, *Atherina boyeri*) are apparently able to detect and avoid acids at levels that do not cause short term harmful effects (Davies 1991).

Forrest et al (2007) tested the toxicity of acetic acid to various fouling organisms and to one non-target species (i.e. green-lipped mussel, *Perna canaliculus*). These authors observed high mean green-lipped mussel attachment (95%, 24 h post-treatment) after 2 minute-immersion in 4 and 8 % acetic acid and high survival (91%, 1 month post-treatment) after 1-4 minute-immersion in 4% acetic acid.

Coutts and Forrest (2005) tested the toxicity of acetic acid to *Styela clava* through immersion in plastic buckets and inside of wrapped pontoons. They observed 100% mortality of *S. clava* at 1% acetic acid after only 10 min. of exposure and almost 100% mortality of non-target species and of *S. clava* but not of Pacific oysters *Crassostrea gigas*, nor calcareous tubeworms (*Pomatoceros terranovae*) at 1% acetic acid after 12 h- exposure.

Environmental fate and decomposition

Biodegradation is the major environmental decomposition process for acetic acid in both soil and water. Acetic acid can be completely degraded via the tricarboxylic acid cycle (i.e. aerobic decomposition into acetate and one hydron) or by methanogenesis (i.e. anaerobic decomposition into methane and releasing heat) (Gottschalk 1985). Aquatic hydrolysis is not significant.

Acetic acid has been noted to leach from biological disposal sites, but it is expected to be readily biodegraded during its migration through soil. Acetic acid does not bioconcentrate (Howard 1990).

Formulations and application methods

In aquaculture settings, gear (e.g. mussel collectors) is usually sprayed with, or immersed in 4-5% acetic acid solution and set out of the water for a few minutes to dry. Coutts and Forrest (2005) successfully injected 1% acetic acid into polyethylene plastic wraps that they used to envelop pontoons infested by *Styela clava* in a protected marina (Auckland's Viaduct Basin, New Zealand). Acetic acid has also been successfully incorporated as the active ingredient in BioBullets (see "Pesticide Application and Containment Methods" Section in Chapter I).

Uses against unwanted mollusks and fouling animals

Controlled experiments and commercial applications indicate that acetic acid is effective at killing a variety of marine biofoulers including invasive tunicates (*Ciona intestinalis*, *Styela clava*, and *Didemnum vexillum*) (Carver, Chisholm et al. 2003; Coutts and Forrest 2007; Forrest, Hopkins et al. 2007; LeBlanc, Davidson et al. 2007; Pannell and Coutts 2007; Denny 2008; Locke, Doe et al. 2009), mussels (*Mytilus galloprovincialis planulatus*) (Lewis and Dimas 2007) and biofouling algae (*Cladophora* sp. and *Undaria pinnatifida*) (Sharp, MacNair et al. 2006; Forrest, Hopkins et al. 2007).

Forrest et al (2007) evaluated the effects of acetic acid to control the dispersal of fouling pests (i.e. tunicates, bryozoans, polychaetes and algae) through the transport of seed stock between marine farming regions in New Zealand. These authors found that acetic acid immersion at 4% concentration was significantly more efficient than at 2%. At 4% acetic acid, only two (*Cnemidocarpa bicornuata* and *Hydroides elegans*) out of 10 indicator species survived the maximum immersion time of 4 minutes, whereas at 2% all indicator species survived and only *Terebellidae* and *Cladophora* sp. were killed given the same immersion time. The tunicates *Ciona intestinalis*, *Cnemidocarpa bicornuata*, *Corella eumyota* were most resilient to the 4% acetic acid treatment, surviving 3 or 4 minutes immersion, while the bryozoans *Watersipora subtorquata* and *Bugula neritina* as well as the tunicates *Botryllus schlosseri* and *Botrylloides leachi* succumbed after only 1 minute immersion. Among the polychaetes, one (*Hydroides elegans*) was very resilient and the other one (*Terebellidae*) was very sensitive. These authors point out that a 4 minute-immersion treatment with 4% acetic acid may not be effective against other pests if their tolerance is comparable to that of structurally and functionally similar taxa, such as in the case of *Ficopomantus enigmaticus* which is similar to *Hydroides*.

Coutts and Forrest (2005) reviewed possible options for eradicating *Styela clava* on a range of substrata (i.e. pontoons, ropes, floats, piles, barges, vessels). In laboratory settings, they registered 100% mortality of *S. clava* after 1 minute of immersion in 4% acetic acid and after 10 minutes of immersion in 1% acetic acid. Subsequent field trials for *in situ* treatments consisted of wrapping piles and pontoons with an impermeable plastic (a precursor to the IM Protector described in Chapter I) to 1) induce *S. clava* mortality over a period of days via the development of anoxic conditions in the encapsulated water and 2) evaluate whether *Styela* mortality could be accelerated within the wraps via the addition of chemicals, including acetic acid. These authors also registered almost 100% mortality of *Styela clava* inside of wrapped pontoons for 12 h at 1% acetic acid.

Ammonium compounds and derivatives

(See Chapters II and IV for more information on Ammonium)

Uses against unwanted mollusks and fouling animals

Polyquaternary ammonium compounds have been used as non-oxidizing molluscicides and compose a number of commercial products specifically approved for the control of zebra mussel (*Dreissena polymorpha*) in closed waters systems, such as in industrial pipes and cooling towers (McEnulty, Bax et al. 2001). Dimethyl-diallyl-ammonium chloride has also been used to control *Dreissena polymorpha* (Blanck et al. 1996).

Ammonia is toxic to the stream snail *Potamopyrgus antipodarum*. However, *P. antipodarum* was relatively intolerant of ammonia compared with other invertebrate taxa reported in the literature (Watton and Hawkes 1984).

A 96-hour acute toxicity tests for ammonia on the brackish stream snail *Potamopyrgus antipodarum* and *P. jenkinsi* using a flow-through system found that juveniles were less tolerant than adults and senescent adults were less tolerant than prime adults (96-h LC50 values of 0.315, 0.49 and 0.85 mg NL-1 unionized N-NH₃) respectively. *Potamopyrgus jenkinsi* was relatively intolerant of ammonia compared with other invertebrate taxa reported in the literature (Watton and Hawkes 1984).

A range of non-oxidizing molluscicides is available in the USA for *Dreissena polymorpha* control. These include; Clamtrol CT2- Betz Chemicals; Calgon H-130 and Calgon Catfloc LS; Macrotrol 7326 Nalco; Mexel 432; Bayer 73; Sal I (Salicylanilide I) and TFM.

Cationic surfactant-based molluscicides DGH/ QUAT were tested against zebra mussels *Dreissena polymorpha* and Asian clams *Corbicula fluminalis* (Bidwell, Farris et al. 1995). *Dreissena polymorpha* experienced significantly higher mortality rates in shorter time periods (6 hours). The aromatic hydrocarbon TCMTB (2-(thiocyanomethylthio) benzothiazole) >1 mg/ kg or PQ1 >2 mg/ kg can induce 100% mortality in zebra mussels and Asian clams more rapidly than exposure to 0.3-0.5 mg/ kg residual chlorine (336-505 hours), they may be more effective molluscicides for the control of bivalve macrofouling in raw water systems (McMahon, Shipman et al. 1993).

Trade names for quaternary ammonium compounds registered for applications in closed water systems include Clam-trol/CT-1, Calgon H-130; Macrotrol 7326; Mexel 432; Bayer 73 and Bulab.

Chlorination

(See Chapters II and IV for more information on Chlorine)

Uses against unwanted mollusks and other fouling animals

Chlorol (10% chlorine) has been used to control *Urosalpinx* gastropods (McEnnulty, Bax et al. 2001).

In estuarine Indian waterworks chlorine gas (1ppm) and chlorine dioxide have been used to successfully control *Brachidontes striatulus*. At 1mg L⁻¹ chlorine residual, 100% mortality occurred in 20 and 24 days for size classes 7 and 25mm in body length, respectively. At 5mgL⁻¹ 100% mortality occurred in ~ 5 and 7 days size classes 7 and 25mm in body length, respectively (Morton, Au et al. 1976; Rajagopal, Nair et al. 1997).

Various forms of chlorine (Sodium hypochlorite NaOCl, Chloramines NH₂Cl, NHCl₂, NCl₃, Chlorine dioxide ClO₂, Sodium chlorite NaClO₂) have been successfully used to control quagga mussel (*Dreissena bugensis*) fouling (McEnnulty, Bax et al. 2001). Mortality of larval *D. polymorpha* occurs at residual chlorine concentrations as low as 0.1 mgL⁻¹ while adults require at least 1 mgL⁻¹ depending on exposure time and water temperature. Zebra mussels are able to sense chlorine and other toxins in their surrounding environment and respond by closing their valves. This enables them to avoid toxic effects for up to 3 weeks (Boelman, Elba A. Dardeau et al. 1997; Rajagopal, van der Velde et al. 2002; Aldrige, Elliot et al. 2006).

In a study that compared the toxicity of chlorine and peracetic acid to the embryos of *Mytilopsis leucophaeata* and *Dreissena polymorpha*, authors found lethal acute toxicity of sodium hypochlorite and peracetic acid to 4 h old embryos of both species; chlorination was found effective against *M. leucophaeata* from a concentration of 0.6 mgL⁻¹ onwards, even at short exposure times (Verween, Vincx et al. 2009).

Electrolysis of seawater to produce chlorine has been successfully used to control fouling (including *Mytilopsis*) on seawater inlets of ships and shore structures under laboratory and field conditions in India (Kalyanasundaram, Ganti et al. 1978).

The black striped mussel (*Mytilopsis sallei*) was eradicated in three locked marinas in Darwin harbor, Australia using sodium hypochlorite (liquid bleach) and copper sulphate. This control attempt was successful because the mussels were in small water bodies separated from the surrounding ocean. Sodium hypochlorite was added to the short channel between the two lock gates separating the marinas from the ocean, to prevent larvae from leaving the marinas alive. Sodium hypochlorite and copper sulfate were added to each marina, and eradication was confirmed by subsequent

monitoring (Bax, Hayes et al. 2002). High ambient temperatures in Darwin meant that it was difficult to maintain residual chlorine concentrations high enough (> 2 ppm) to guarantee a complete kill in a reasonable time (especially in laboratory trials). Copper sulphate was used instead of, or in addition to, the chlorine to ensure the complete the kill of mussels. *Mytilopsis leucophaeta* is also killed by chlorine applications, although the species is more tolerant than blue mussel *M. edulis* and *D. polymorpha* (Rajagopal, Nair et al. 1997).

Continuous chlorination at a residual chlorine level of 0.1-0.25 mgL⁻¹ had no effect on settlement of *M. edulis*. Mortality of larval *M. edulis* occurs at residual chlorine concentrations as low as 0.1 mgL⁻¹ while adults require at least 1 mgL⁻¹ depending on exposure time and water temperature (Jenner, Taylor et al. 1996; Jenner, vanderVelde et al. 1997; Rajagopal, Nair et al. 1997). Continuous chlorination at residual chlorine levels between 0.2-0.5 mgL⁻¹ delayed settlement for 30% of *Perna viridis* larvae (Masilamoni, Jesudoss et al. 2002; Rajagopal, Van der Velde et al. 2003).

According to Jenner and Janssen-Mommen (1993) and citations therein, *Corbicula fluminea* could be killed by continuous chlorination in 2-3 weeks at total residual chlorine concentrations of 0.5 mgL⁻¹ and water temperatures of 20-25 °C.

Continuous chlorination at a low concentration (0.5 mgL⁻¹) has been used to successfully control *Limnoperna fortunei* fouling in raw water supplies in Hong Kong. Applications of 1.0 mgL⁻¹ chlorine over a period of several days at 2-3 month intervals has been shown to be an effective way for keeping industrial pipes and conduits clear, although dense fouling may require an initial high dose (e.g. 200 mgL⁻¹). Intermittent chlorination is generally ineffective in preventing mussel settlement and growth, as mussels that settle between the chlorine pulses seem to be able to resist subsequent exposures to chlorine. Settlement and recolonization of the system by newly metamorphosed mussels can be prevented if chemicals are applied after and during spawning periods (Morton, Au et al. 1976; Ricciardi 1998). A selective summary of the details of chlorination of various mussel species in power plant biofouling control is given in (Rajagopal, Nair et al. 1997).

Potassium chloride (KCl) has been successfully used to control zebra mussel (*Dreissena polymorpha*) utilizing an alternative application method, the so-called BioBullet technology (see Chapter I). This method consists of the encapsulation of an active ingredient in microscopic particles of edible material (Aldrige, Elliot et al. 2006). However, potassium chloride was associated to metamorphosis in the marine gastropod mollusk *Crepidula fornicata* (L.) following natural or triggered metamorphosis (Eyster and Pechenick 1988).

In field trials, 10% chlorine solutions seemed successful to control the Atlantic oyster drill *Urosalpinx cinerea* on oyster beds in the UK (Hancock 1959).

Studies have found that mussels treated with chlorine concentrations of 4.43 mgL^{-1} for 49 hours were capable of making a recovery but failed to recover after a 24 hour exposure to a chlorine concentration of $8\text{-}40 \text{ mgL}^{-1}$ (Rajagopal, Nair et al. 1996).

Iron phosphate

General information

Iron phosphate (i.e. ferric phosphate) is a naturally occurring chemical in soils and is registered by the USEPA as a micide for terrestrial use only. This pesticide is registered to be applied in the outdoors (ornamental, lawn and garden use), as well as in food crops (vegetables, berries, fruit trees including citrus) and indoors, in greenhouses (USEPA 1998).

Mode of action

Main mode of action is stomach poisoning and does not include dehydration. The exact mode of action of these stomach poisons is not fully elucidated, but iron appears to be deposited in the digestive gland and body wall which leads to reduced levels of feeding and, potentially, slug death (Triebkorn, Henderson et al. 1999).

Selectivity:

According to the label of SLUGGO, a 1% iron phosphate terrestrial molluscicide currently in the market, the product kills *Deroceras reticulatum* (field slug), *Deroceras laeve* (Smooth slug), *Arion subfuscus* (Dusky slug), *Arion circumscriptus* (Gray garden slug), *Arion hortensis* (Black field slug), *Arion rufus* (Large red slug), *Arion ater* (Large black slug), *Limax flavus* (Spotted garden slug), *Limax tenellus* (Slender slug), *Ariolimax columbianus* (Banana slug), *Helix* spp., *Helicella* spp., and *Cepaea* spp., among others. Very little is known about the effects of iron phosphate formulations on non-target fauna. Pelleted iron phosphate seems to cause deleterious impacts on the survival and behavior of the earthworm *Lumbricus terrestris* (Langan and Shawa 2006).

Toxicology

During the registration process for iron phosphate for the control of terrestrial snails and slugs, a number of ecological effects toxicology data requirements were waived based on the known lack of toxicity of iron phosphate to birds, fish and non-target insects, in association to its low solubility in water, conversion to less soluble form in soil, and its use pattern (soil application and in small rates). An acute oral toxicity study in Bobwhite quail (NOEL & LD50 greater than 2000 mg/kg) indicated that iron phosphate was practically nontoxic to avian species. Based on these factors, the data requirements for the toxicity studies in Mallard duck, rainbow trout, freshwater invertebrates, and nontarget insect/honeybees were waived. The USEPA considered exposure to ground-feeding nontarget insects and earthworms likely. Studies involving ground beetles, rove beetles and earthworms demonstrated that the product will not affect these organisms at up to two times the maximum application rate (USEPA 1998). Also, the USEPA does expect adverse effects to humans. Iron phosphate is considered GRAS (generally regarded as safe for food use).

Formulations and Application methods

Sold as a pellet formulation (1% FePO) in the U.S.A. and Canada as SLUGGO for terrestrial use only.

Uses against unwanted mollusks and other fouling animals

The effect of NEU1165M Slug and Snail Bait™ (active ingredient is iron phosphate at 1%) on *Pomacea canaliculata* was tested by Jackson and Santo (2004) from the Hawaii Agriculture Research Center (HARC) and compared with the effects of neem and papaya extracts. Their results registered that the iron phosphate bait had little or no effect on the target species. The apple snails apparently ingested the baits, but mortality was similar to that registered in the control experiment. The authors concluded that further studies are needed to determine the true efficacy of ferric phosphate in this system against apple snails.

Lime compounds and derivatives

(See Chapters II and IV for more information on lime compounds and derivatives)

Uses against unwanted mollusks and other fouling animals:

Hydrated lime (calcium hydroxide) solutions have been tested in the combat of *Crassostrea gigas* and *Codium fragile*.

Quicklime (calcium oxide) is mostly insoluble and has been successfully deployed in porous as a barrier control around commercial oyster farms to control sea stars *Asterias amurensis*, *Asterias forbesi* and *A. vulgaris*, oyster drills (*Urosalpinx*, *Eupleura*) and other fouling species since the turn of the century. It has been used in Korea, Canada and the US (MacKenzie 1977). However, C.L. Goggin (CRIMP; unpublished data in Thresher et al. 1998), reports that sea stars must be in contact with the lime for lengthy periods (>5 hours) in order for it to be effective, and in a relatively two dimensional habitat where they are unable to evade the lime (Thresher et al. 1998).

In Connecticut, US, quicklime was spread at a rate of 6.75 metric tons hectare⁻¹ (<6 m depth) on dormant oyster beds to kill *A. forbesi*. Repeated applications of quicklime on an oyster bed at weekly intervals for 5 weeks of June-July in the US kept the upper side of the oysters clean of fouling organisms and provided a favorable surface for settlement of oyster larvae (MacKenzie 1977). Sea stars settle or crawl over the lime and stop feeding very shortly after body contact. Lesions form after 24-48 hours and sea stars usually die within two weeks. Sea stars injured by the lime are more prone to be attacked by crabs and other animals, increasing their mortality rate (Loosanoff 1961). While quicklime has only slight effects on mollusks such as *Mercenaria mercenaria* (Loosanoff and Engle 1942) it has severe effects on crabs.

Hydrated lime has been used for years to control predatory starfish on mussel seed (spat) collectors in Prince Edward Island (PEI) estuaries, in Canada. The method, which consists of briefly immersing each collector in a trough filled with a saturated solution of hydrated lime in seawater, has been adapted for tunicate management on mussel socks and other aquaculture gear (Locke, Doe et al. 2009).

MacNair and Smith (2000) found that immersing oyster spat collectors in a 4% hydrated lime solution for 1 minute effectively killed *Molgula* sp.. Mortality of mussels following immersion of a mussel sock in a trough is up to 10-15%; and mortality increases if the valves of the mussels are not closed during treatment (Locke, Doe et al. 2009).

Hydrated lime solutions caused significant mortalities to *Crassostrea gigas* (Rikard and Wallace 1997; Cigarria et al. 1998).

Hydrated lime has been successfully used to control zebra mussel (*Dreissena polymorpha*) utilizing an alternative application method, the so-called BioBullet technology (see Chapter I). This method consists of the encapsulation of an active ingredient in microscopic particles of edible material, and has been approved for use in drinking water systems in the UK (Aldrige, Elliot et al. 2006).

Neem and papaya extracts

(See Chapter II for more information on Neem)

Use against unwanted mollusks and other fouling animals

In a research undertaken by the Hawaii Agriculture Research Center (HARC) in 2001 and 2002, neem (*Azadirachta indica*) and papaya (*Carica papaya*) extracts were shown to be toxic to *Pomacea canaliculata*. The active ingredient in papaya was shown to be the proteolytic enzyme papain. The active ingredients in the neem extract were thought to be the azadirachtins, but this was not demonstrated due to a lack of suitable purified materials (Jackson and Santo 2004).

According to Jackson and Santo (2003), previous experiments had shown that the latex from green papaya had a significant effect on snail mortality, with 100% mortality within 48 hours after application in an aquarium system in the laboratory. Latex was obtained by scoring green papaya fruits and collecting the milky latex that exuded. The latex was dried at room temperature and then pulverized to form a white powder. The neem and papaya fruits had not been exposed to pest control chemicals and therefore the potential of confounding any results due to chemical contamination was avoided. These products were applied to experimental taro patches in different proportions, combined and individually.

While the papaya extracts had been effective in the laboratory aquaria tests, the highest mortality observed was 54% for a papaya/ neem combined treatment. Neem alone resulted in a 54% maximum mortality, while papaya extract alone resulted in 32% maximum mortality (12% mortality was observed in the control plot) (Jackson and Santo 2004). The authors explained that a number of factors could have contributed to this difference, including the fact that in the field there is layer of muddy, flocculant soil under the water. This soil has a high organic content, which may have adsorbed the non-specific protease papain, in high concentration in the papaya extract, attenuating its effect on the snails.

Potassium permanganate

(See Chapter II for more information on potassium permanganate)

Use against unwanted mollusks and fouling animals

Potassium permanganate has been tested as a control for zebra mussels (*Dreissena polymorpha*) with limited success (Van Benschoten et al. 1993; Matisoff et al. 1996; Boelman et al. 1997) and some success against adult zebra mussel at 2.0 mg/ L. It also inhibited veliger settlement at 1.0 mg/ L (San Giacomo and Wymer 1997).

Limited success was observed when potassium permanganate was tested as a control for the gastropod *Urosalpinx cinerea* in oyster beds in the UK (Hancock 1959).

Saponins

(See Chapter II for more information on Saponins)

General information

Endod is the common name given to the bush *Phytolacca dodecandra* in Ethiopia, where its berries have a long history of use as a soap substitute. Elsewhere, this plant is commonly referred to as “soapberry”. The molluscicide properties of Endod were noticed in the mid-1960’s by Aklilo Lemma. This researcher first applied endod-derived saponins to control freshwater mollusks (e.g. *Biomphalaria* spp., *Bulinus* spp. and *Oncomelania* spp.) that transmit tropical parasitic diseases such as schistosomiasis. Thirty years later, Lemma, in association with researchers from the University of Toledo, Ohio, began to explore the use of endod to control zebra mussels (*Dreissena polymorpha*). They developed a method to control zebra mussel invasions by putting these organisms in contact with a butanol extract of grinded dried endod berries, and found that at a dose higher than 15 mg L⁻¹, endod is lethal to adult zebra mussels, while at lower doses it prevents their adhesion and aggregation (Lee, Lemma et al. 1993). Two U.S. patents have been awarded for its use as a molluscicide (Lee, Fraleigh et al. 1993; Lee, Fraleigh et al. 1994), but the product is not a registered pesticide with the EPA. Some toxicity studies of this product have been carried out and are discussed in the sections below.

Toxicology

It has been reported that the molluscicidal activity in Endod disappeared in 1 or 2 days in field trials in Africa, and repeated application was required to kill snails that carried schistosoma larvae. This decrease in molluscicidal activity due to biodegradation has been interpreted as an indication that Endod toxicity to nontarget organisms would elicit a minimal risk. According to these studies, unlike other molluscicides which may bioaccumulate in the

foodchain, Lemmatoxins disappear after they exert lethal effects to zebra mussels and their insoluble byproducts subsequently decay.

Environmental fate and decomposition

It has been proposed that Endod's decomposition and loss of toxicity are due to the breaking of the glycosidic bonds by enzymatic procedures since glycosidase is a common cellular enzyme, and that the nontoxicity on mammals may also be due to enzymatic degradation of Endod in animal systems (Lambert, Temmink et al. 1991; Hietanen 1996). A more recent toxicological study has supported these observations, and although it did not fully clarify the biochemical processes involved in it, it has confirmed endod's potential as a safe method to control freshwater snails, specially due to its low acute toxicity (Hietanen 1996). Nevertheless, further tests, for example, absorption/adsorption tests with sediments, must be done before Endod is accepted as a control agent for zebra mussels in water intakes of North America. In addition, there has been practically no systemic study of mechanism of action of Lemmatoxins at cellular or physiological levels (Lee, Lemma et al. 1993), which would be crucial to fully understand long-term impacts of the application of these saponins at the scale that is necessary to control invasive species.

Use against mollusks/ fouling animals

The use of saponins has also been considered as a method to control Golden apple snails (*Pomacea canaliculata*). Extracts of soapnut (*Sapindus mukorossi*) showed molluscicidal effects against the golden apple snail with LC₅₀ of 85, 22, and 17 ppm after treating 24, 48, and 72 h, respectively. Bioassay-directed fractionation of *S. mukorossi* resulted in the isolation of one new hederagenin-based acetylated saponin, along with six known hederagenin saponins, all displaying icidal activity and causing 70-100% mortality at 10 ppm against the golden apple snail (Huang, Liao et al. 2003). Unfortunately, no references to further studies or tests with these extracts have been found.

Another source of saponins with molluscicide properties is the already mentioned tea seed cake powder, a product widely used in Asian countries to control not only apple snails, but also to kill undesired fish in shrimp ponds..

In 2007, a set saponins extracted from quinoa (*Chenopodium quinoa*) and with molluscicide properties was described and evaluated (San Martin 2007). Quinoa is a pseudocereal extensively cultivated and consumed in Bolivia and Peru. The external husk of the grains is removed prior to human

consumption due to the bitter taste imparted by their high saponin content (predominantly bidesmosidic saponins), and constitutes a by-product with no commercial value. When tested against golden apple snails *Pomacea canaliculata*, quinoa husks showed no activity up to 121 ppm product (approximately 35 ppm saponins). To increase their icidal properties, the husks were treated with alkali to convert bidesmosidic saponins to more active monodesmosides. This product killed 100% of the golden apple snails under laboratory conditions in 24 h at approximately 33 ppm product. The few toxicity tests performed so far showed that these saponins are not toxic to fish (*i.e.* goldfish and tilapia) up to 54 ppm product (San Martín, Ndjoko et al. 2008). The product was also tested under field conditions in rice fields of Northern Argentina, and the Philippines (Joshi, Martín et al. 2008), showing similar promising results both in terms of mortality of the target-species and of the low environmental toxicity.

A preliminary LC/ESI-MS/MS study (San Martín, Ndjoko et al. 2008) revealed that the husks contained a mixture of known and novel bidesmosidic and monodesmosidic saponins. However, the alkali treated husks did not contain monodesmosidic saponins as the authors of this study expected. Instead, large molecular weight saponin derivatives probably formed between the saponins and other compounds present in the quinoa hulls under alkaline conditions were found. However, a relationship between product efficacy and a higher content of monodesmosidic saponins could not be established. These authors attributed that to the complex nature of these compounds, and suggested that the icidal properties of the quinoa-derived saponins are probably related to the formation of more hydrophobic compounds after alkaline treatment, which would have a higher affinity with the cholesterol present in apple snails' gills (San Martín, Ndjoko et al. 2008). Applications for US and international patents for the above mentioned product's application as a molluscicide have been filed in 2007 and claim that these modified saponins are effective not only against apple snail species, but against other freshwater mollusks, including zebra mussels (San Martín 2007; San Martín 2007).

Since the discovery of Endod in 1965, there has been a series of studies on the chemistry, toxicity, and epidemiology of the so-called Lemmatoxins. A consortium of laboratories in North America and Europe was carried out in the early 1990's (Lambert, Temmink et al. 1991) undertook a Tier 1 EPA study with the Organization for Economic Cooperation in Development (OECD) in accordance with Good Laboratory Practice guidelines and indicated that Endod at 10 ppm was not toxic to mammals, although it was lethal to the snails *Biomphalaria glabrata* and *Bulinus truncatus*. These tests included a 28-day oral administration to rats and irritant tests for eyes and skin. An eye irritant test showed that Endod irritates eyes; therefore, eye

protection should be used during preparation and application of the Endod powder. Together with previous tests (Lemma and Ames 1975), which indicated that Endod was neither mutagenic nor carcinogenic, Endod has been considered to be safe in large systemic scale field tests in African streams where people perform their daily chores, in addition to being the drinking water sources for many (Lee, Lemma et al. 1993).

Equally limited are toxicological assessments of *Chenopodium quinoa* saponins. So far, apparently only one study of toxicity to non-target freshwater species was performed. The results from this test was presented concomitantly with the novel molluscicide itself, the alkali treated quinoa husks product, and it was very limited both in terms of species evaluated (i.e. only two, goldfish and tilapia) and it terms of potential chronic effects (San Martin, Ndjoko et al. 2008).

In order to grant a biopesticide registration for *C. quinoa* saponins to be used as a seedlings protectant, back in 2005, the USEPA had to review data requirements as prescribed by FIFRA Section 3(c)(5). This review included an evaluation of mammalian toxicology requirements and ecological effects data, and concluded that saponins of *C. quinoa* adequately satisfied current guideline requirements. It is important to highlight, however, that the saponins under evaluation in that instance where the naturally-occurring unmodified saponins extracted from raw quinoa, as opposing to the alkali treated quinoa hulls product that has been proposed as a molluscicide agent. In fact, greatly due to the non-synthetically modified nature of this product, the USEPA waived or substituted many of the toxicity test requirements that are usually intrinsic to this type of review. Such exceptional lenience was given to this application under the argument that 1) the technical grade active ingredients (TGAI, in this case the unmodified quinoa saponins) are naturally occurring; 2) applications of these saponins covered by the registration are “non-food uses”; 3) the TGAI as used in the end product does not require a tolerance; 4) (unmodified) saponins from *C. quinoa* (according to a review of the literature, not new tests) have a very low mammalian toxicity ; 5) no edible commodity was presented at the time of the pesticide product application; and 6) no detectable product residues will be present at the time of harvest, as residues of (unmodified) saponins from *C. quinoa* rapidly degrade within a few days of application (USEPA 2005).

Of all saponins, quinoa-extracted saponins (i.e. triterpene bidesmosidic glycosides of oleanolic acid, hederagenin, and phytolaccagenic acid) are the only ones known to be active compounds of a pesticide registered in the U.S., under the trade name Heads Up[®] Plant Protectant (USEPA 2005). However, these quinoa-extracts are not the ones known to be toxic to mollusks.

Comparative Analysis

In general, most projects for aquatic control of mollusks are aimed at eliminating mollusks that serve as vectors and/ or hosts for parasites and other infectious agents (e.g. several snail species of the Planorbidae family that serve as host for parasitic trematodes of the *Schistosoma* genus) followed by those carried out to prevent or reduce macrobiofouling (e.g. infestations of *Dreissena polymorpha*) in industrial sites, water supply systems, hydroelectric companies and navigation waterways.

Other control programs for aquatic mollusks include those intended to reduce populations of mollusks that cause negative impacts to agriculture (e.g. *Pomacea canaliculata*, which severely damages taro and rice farms), and those with the purpose of controlling mollusk infestations that may affect ecosystemic functions (e.g. *Corbicula fluminea* invasions, which can alter trophic and nutrient dynamics of aquatic systems, and to displace native bivalves). In addition, the use of chemicals may also be necessary for rapid-response and prevention programs, to sterilize hulls of vessels or other aquatic structures that could serve as source of propagules for new infestations.

The use of chemical methods to control fouling in closed systems, such as industrial facilities, pipes, and dams, is often successful and relatively safe. Various options exist in the market for this kind of control, including the traditional oxidizing methods, primarily chlorination, and the application of modern non-oxidizing quaternary ammonium compounds.

In contrast, the application of chemical molluscicides in open and natural aquatic sites is limited by several factors related to the low selectivity of the majority of the molluscicides and to potentially harmful by-products that may be generated by these chemicals depending on variable environmental conditions. For these reasons, the number of active ingredients currently registered for use in aquatic ecosystems like ponds, lakes and rivers is significantly restrictive. Only two active ingredients (i.e. copper and niclosamide) are registered by the USEPA for the control of mollusks in open aquatic environments and these registrations are specifically meant for the control of “freshwater snails which carry the vectors for diseases which affect fish and humans”(USEPA 1999; USEPA 2006).

Currently, two chemicals seem to be suitable for rapid response actions, both for the eradication of early-detected mollusk/ fouling introductions as well as for the disinfection of hulls and other structures that could contaminate aquatic systems: copper sulphate and chlorine. These two active ingredients were successfully used in the eradication of *Mytilopsis sallei* from Darwin Harbor, Australia (Bax, Hayes et al. 2002), and have both been subjected to extensive testing and review.

Unfortunately, the application of these ingredients to marine waters represents substantial risk to non-target species. Also, there are problems associated to the accumulation of copper in ambient sediments and the formation of persistent chlorinated organic by-products (e.g. trihalomethanes, haloacetic acids and chlorite) and chloramines upon reaction with organic compounds. Alternative application methods and containment strategies that could limit the dispersal of these chemicals in the water could minimize the above mentioned side-effects.

For instance, fouled vessels in New Zealand have been wrapped in water with a heavy duty, fiber reinforced PVC sheath with inflatable floatation collars plastic in a configuration that suits the majority of cruising yachts, and fishing boats (Shields and Coutts 2009). This technology, so-called IMProtectorTM, has showed promising results in tests that attempted to eliminate fouling species such as *Didemnum vexillum* (Coutts and Forrest 2007). The major mechanism envisioned by the IMProtector's developers is the creation of anoxic conditions, but mortality could be accelerated through the addition of chemical agents to the encapsulated seawater, including chlorine and copper. Coutts & Forrest (2007) have also tested acetic acid as an adjuvant for killing hull fouling organism.

Another innovation that could reduce the risks associated with the use of copper and chlorine for rapid-response is the BioBullet technology which consists of the microencapsulation of active ingredients in selected edible materials that can be filtered by aquatic mollusks (Aldrige, Elliot et al. 2006). The size, surface texture and composition of the edible material can be adapted to match the feeding habits of the target species in order to minimize risks to non-native species. This technology has been adapted for the control of zebra mussels using food grade active ingredients (i.e. acetic acid, citric acid and calcium hydroxide) with approval for use in European drinking waters and is currently being adapted for the control of *Didemnum vexillum* in the US (David Aldridge, BioBullets developer, Pers. Comm.).

Further research must be done to evaluate the possibility of combining the IMProtector and BioBullet technologies with the use of copper, chlorine, acetic acid and also niclosamide for rapid response actions. In the case of chlorine, neutralization with sodium sulphate or sodium thiosulfate is possible, and should be considered. The utilization of these chemicals with alternative application methods, containment strategies and neutralization techniques should also be tested in combination with non-chemical control options, such as hot water or hypersaline spraying and mechanical removal.

It is important to highlight that these chemicals are not currently registered for use in the marine environment, thus special permits would have to be obtained for this type of uses (i.e. for disinfection of vessel hulls and immediate eradication of new introductions).

Niclosamide seems to be an effective active ingredient against mollusks and other fouling groups. It rapidly degrades into non-persistent and non-toxic elements and has low toxicity to most other aquatic groups. These characteristics have made possible for niclosamide's registration for control of sea lampreys in the Great Lakes, and make it a good candidate for rapid response efforts in other aquatic systems.

Also promising are the results from research with saponins, especially endod and quinoa extracts. The possibility of using these ingredients, as well as niclosamide for the control of apple snail infestations in taro patches should be further investigated. The eradication of well established invasors, such as the apple snail is unlikely, but long-term control programs could ameliorate the negative impacts of these nuisance species.

Finally, results from the research done with iron phosphate, neem and papaya extracts are inconclusive and potassium permanganate seems to be inadequate for the control of aquatic mollusks and fouling species.

The problems associated with the infestation of golden apple snails *Pomacea canaliculata* in taro farming areas of Hawaii are well documented (Levin 2006).

Available evidence suggests that most saponins that can be used as molluscicides to control aquatic invasive species are not likely to cause harm to human health and are expected to cause little impact to the environment (the exception being tea seed cake powder). This evidence takes a special weight when contrasted with the well

documented potential harmful effects associated with the use of synthetic molluscicides (e.g. niclosamide, methaldehyde and copper). Nevertheless, the overall lack of toxicological studies, especially regarding chronic effects of these compounds, and the poor understanding of the mode of action of most saponin presented here are a matter of concern, and should be scrutinized before field tests can be performed.

The main disadvantages of using chlorine compounds for the control of unwanted species are potential direct harmful effects to non-target species and, in the cases of chlorination with gas or bleach, the formation of ecotoxic trihalomethanes (THMs) and chloramines by reaction with organic material in the water (Clearwater, Hickey et al. 2008; Gregg, Rigby et al. 2009 and references therein).

Chapter IV: Aquatic Herbicides, Algaecides, Bactericides and Viruscides

Currently, there are 12 active ingredients registered by the USEPA for chemical control of aquatic plants and algae in natural and open water systems, such as ponds, lakes and streams: eight herbicides, one algaecide, two compounds with both algaecidal and herbicidal properties, and one combination of dyes that will negatively impact algae and plants through shading. Many other active ingredients are registered for algal control in industrial, commercial and other closed systems, such as swimming pools, holding tanks, cooling water and other industrial closed systems; these products are outside the scope of this review, unless they were identified as promising for the control of AIS in open water systems. These algaecides are presented in the “Selected Non-Registered Herbicides and Algaecides” Section below.

Herbicides are generally categorized as systemic versus contact herbicides, and broad spectrum versus selective herbicides. Systemic herbicides are taken up within the plant tissues resulting in the death of the entire plant, while contact herbicides affect only those surfaces of the plant that are exposed. Broad spectrum herbicides kill many different plant species, as opposing to selective herbicides which kill only targeted plant species.

Aquatic plants can be morphologically grouped as free floating (e.g. waterhyacinth *Eichhornia crassipes*; giant Salvinia *Salvinia molesta*), as rooted emergent (e.g. alligatorweed *Alternanthera philoxeroides*, spartina *Spartina alterniflora*), or submersed (e.g. hydrilla *Hydrilla verticillata*; Eurasian watermilfoil *Myriophyllum spicatum*). Management strategies vary substantially with these different growth forms.

Herbicides formulated for aquatic use do not have surfactants, so the applicator will have to add a surfactant appropriate for aquatic use when applying to the aerial portions of floating- leaved and emergent plants. For submersed plant applications, no surfactants are required. For the herbicide to be effective, the plants must be in contact with an adequate amount of herbicide for a long enough period of time to be effective. For contact herbicides, contact times of 6 to 12 hours is often sufficient; whereas some of the systemic herbicides will require contact times ranging from 12 hours to 60 days.

Knowledge of the water exchange characteristics of the treatment site is critical for a proper herbicide treatment (Poovey and Getsinger 2005; Madsen 2006).

Hawai'i has directly experienced the high costs and difficulties associated with control efforts of aquatic invasive plants. For instance, during the latest infestation of giant salvinia (*Salvinia molesta*) in Lake Wilson/Wahiawa Reservoir, O'ahu, this invasive water fern covered virtually the entire surface of the 300-acre reservoir, and clean-up costs approached one million dollars. Other species of freshwater aquatic plants that have been proved to be invasive include waterhyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*) and elodea (*Egeria densa*) are also present in Hawaii (Shluker 2003).

Marine invasive non-indigenous seaweeds present in Hawaii include spiny seaweed *Acanthophora spicifera*, *Avrainvillea amadelpha*, *Gracilaria salicornia*, *Gracilaria tikvahiae*, *Hypnea musciformis* and *Kappaphycus* spp. (Eldredge and Smith 2001). The widespread distribution and impacts of these algae in Hawaii are well documented in the literature (Russell 1987; Rodgers and Cox 1999; Woo 1999; Smith, Hunter et al. 2002).

Next, I review all active ingredients that compose herbicides and algaecides registered by the USEPA for use in open and natural aquatic systems (e.g. ponds, lakes, rivers, wetlands). I also review promising active ingredients that are not presently registered for the control of aquatic vegetation or algae. Results from field and laboratory experiments that tested the effects of these active ingredients to control of seaweeds, and coastal vegetation are reported. At the end of this chapter, I offer a comparative analysis of the chemical methods taking into consideration individual pros and cons, as well as their selectivity, efficacy, legal status and suitable environmental settings.

USEPA-registered Aquatic Herbicides and Algaecides

2,4-D (2,4-dichlorophenoxyacetic acid)

General information

2,4-D has been identified by the USACE as the most cost-effective manner to control broadleaf eudicotyledon plants (Getsinger 2004; USEPA 2005; Washington State Department of Ecology 2008).

Mode of action

2,4-D is a relatively fast-acting systemic herbicide that inhibits normal plant growth by interfering with the process of cellular division. It is absorbed by both leaves and roots, and translocated to actively growing areas. Thus, better results are attained if it is applied during periods of active growth (AERF 2008).

Selectivity

2,4-D products are relatively selective to broadleaf eudicotyledon plants and used to control invasive weeds that fall into this category. Susceptible weeds include water milfoil (*Myriophyllum spp.*) and water stargrass (*Heteranthera dubia*), while slightly to moderately resistant weeds include bladderwort (*Utricularia spp.*), white water lily (*Nymphaea spp.*), yellow water lily (*Nuphar spp.*), water shield (*Brasenia spp.* w/ "s"), water chestnut (*Trapa natans*) and coontail (*Ceratophyllum Demersum*) (Applied Biochemists 2008).

Toxicology

2,4-D is a General Use Pesticide in the US. In 2005, the EPA reviewed all toxicity studies submitted for 2,4-D and determined that the active ingredient was suitable for reregistration. EPA considered that 2,4-D generally has low acute toxicity via the oral, dermal and inhalation routes of exposure. Also, that 2,4-D is not a skin irritant, nor a skin sensitizer. Although the 2,4-D ester forms are not eye irritants, the acid and salt forms are considered to be severe eye irritants.

There is no evidence that 2,4-D bioaccumulates to significant levels in mammals or in other organisms (Kamrin 1997). In long-term studies, at dose levels above the threshold of saturation for renal clearance, 2,4-D is toxic to the eye, thyroid, kidney, adrenals, and ovaries/testes (USEPA 2005). In humans, prolonged breathing of 2,4-D causes coughing, burning, dizziness, and temporary loss of muscle coordination. Evidence suggests that if 2,4-D causes reproductive, teratogenic, or mutagenic effects in animals, this only occurs at very high doses (Kamrin 1997; USEPA 2005), and according to the RED for 2,4-D, neither acute, short-term or long-term

aggregate risk are of concern because they are beyond the thresholds set for these standards (USEPA 2005).

Carcinogenic effects of 2,4-D are not clear (Kamrin 1997). The EPA Cancer Peer Review Committee (1996) identified 2,4-D as “not classifiable as to human carcinogenicity”. In 2007, the EPA confirmed this decision by stating that existing data do not support a conclusion that links human cancer to 2,4-D exposure (USEPA 2007). However, the International Agency for Research on Cancer (IARC) has classified 2,4-D among the phenoxy acid herbicides (2-methyl-4-chlorophenoxyacetic acid and 2,4,5-T) in class 2B for carcinogen and possibly carcinogenic to humans (WHO 1987).

2,4-D is applied to water bodies in the acid, amine or BEE forms for control of aquatic weeds. Adults and children may be exposed to 2,4-D while swimming in these bodies of water. Because 2,4-D concentration in water can vary depending upon the application rate and site conditions, the EPA requested Maximum Swimming Water Concentrations (MSWCs) to be calculated. The Acute MSWC of 9.8 ppm for exposures to 2,4-D acid or amine is greater than the master label application rate of 4.0 ppm, therefore, acute exposures to acid or amine are not of concern. The short term MSWC of 3.6 ppm for short term exposures to acid or amine is also not of concern because some dissipation or dispersion is likely to occur which would cause the 7-day average of 2,4-D concentrations to be less than 3.6 ppm (USEPA 2005).

The MSWCs for 2,4-D BEE are less than the master label application rate of 4 ppm, but they are unlikely to be of concern because 2,4-D BEE degrades rapidly by microbial and abiotic hydrolysis. According to the EPA, although their calculations indicate that the risk estimates 2,4-D BEE are conservative, a 24-hour post-application restriction on swimming is necessary to ensure the safety of children swimming in treated water (Kamrin 1997).

2,4-D is slightly toxic to wildfowl, and slightly to moderately toxic to birds. Some formulations of 2,4-D are highly toxic to fish while others are less so. Limited studies indicate a half-life of less than 2 days in certain fish and oysters species. Other studies, however, indicate that 2,4-D may affect embryonic development and have drastic effects on larval growth and survival of *Crassostrea gigas* as well as on other species. Furthermore, post-treatment fish kills may occur due to oxygen depletion resulting from decomposition of dead plant material. For this reason one should not treat an entire pond or lake at one time, but instead treat strips or quadrates and wait at least two weeks before follow-up treatments are made (EXTOXNET 1995).

Freshwater amphibian studies conducted on frog tadpoles (*Rana pipiens*) indicate that 2,4-D acid, 2,4-D DMA, and 2,4-D EHE are practically non-toxic to tadpoles. Acute toxicity of 2,4-D acid and amine salts to freshwater aquatic invertebrates ranges from a LC50 of 25 to 642.8 mg aeL-1 (slightly toxic to practically non-toxic). The freshwater toxicities of the esters range from 2.2 mg aeL-1 for the 2,4-D IPE to 11.88 mg aeL-1 for the 2,4-D EHE (moderately toxic to slightly toxic). Acute toxicity of 2,4-D acid and amine salts to marine invertebrates range from an LC50 of 49.6 for 2,4-D IPA to 830 mg aeL-1 for 2,4-D DMA (slightly toxic to practically nontoxic). The marine invertebrate LC50 s range from >0.092 to >66 mg aeL-1 for the 2,4-D esters (highly toxic to practically non-toxic). These toxicities indicate that the esters are more toxic than the acid and amine salts. Although acute data were missing for some of the amine salts, the EPA decided that these studies were not required because none of the risk quotients exceeded the aquatic levels of concern for the acid amine salts (USEPA 2005).

Chronic toxicity tests for freshwater and estuarine/marine invertebrates were performed on 2,4-D acid, 2,4-D DEA, 2,4-D DMAS, and 2,4-D BEE. The toxicity ranged from a no observed effect concentration (NOEC) of 16.05 mg aeL-1 for 2,4-D DEA (survival and reproduction) and 79 mg aeL-1 for the 2,4-D acid (number of young). Chronic freshwater NOEC was 0.20 mg aeL-1 for the 2,4-D BEE (survival and reproduction). No freshwater or marine chronic toxicity data for any of the other 2,4-D esters was examined by the EPA. Although an estuarine/marine invertebrate life-cycle toxicity test using the Technical Grade of the Active Ingredient is required to establish the toxicity of products containing the 2,4-D acid, salts, and amines, a chronic study was not be required by the EPA. Instead, the data from the freshwater invertebrate studies was bridged to the estuarine/marine invertebrates for the 2,4-D acid and amine salts based on the logic that the risk quotients for the freshwater chronic studies were well below the levels of concern, and the chronic risk for estuarine/marine invertebrates was therefore expected to be low (EXTOXNET 1995).

In a controlled experiment that examined the impact of selected pesticides on the biodiversity of aquatic communities containing algae and 25 species of animals, 2,4-D had few effects on any species or trophic group in the entire community during the 14-day experiment (Relyea 2005). This was consistent with past toxicity studies that have found relatively high LC₅₀ 96-h values for 2,4-D, including 45mgL-1 for lake trout (*Salvelinus namaycush*), 301 mgL-1 for American eels (*Anguilla rostrata*), and 363–389 mgL-1 for cladocerans (*Daphnia magna*).

Environmental fate and decomposition

In aquatic environments, microorganisms readily degrade 2,4-D. Rates of breakdown increase with increased nutrients, sediment load, and dissolved organic carbon. Under oxygenate conditions its half life is of 1 to several weeks. 2,4-D has low soil persistence (half-life in soil is less than 7 days). Soil microbes are primarily responsible for its decomposition. Despite its assumed short half-life in soil and in aquatic environments, the compound has been detected in groundwater supplies in Canada, and in surface waters throughout the U.S. (Kamrin 1997).

Formulations and application methods

There are two formulations of 2,4-D approved for aquatic use and many forms or derivatives of 2,4-D including, esters, amines, and salts. The granular formulation contains the low-volatile butoxy-ethyl-ester formulation of 2,4-D (AquaKleen® and Navigate®). The liquid formulation contains the dimethylamine salt of 2,4-D (DMA*4IVM). Granular formulations are reported as effective to control waterhyacinth, Eurasian watermilfoil, water stargrass, bladderwort, lillies, spatterdock and coontail, while the liquid formulation seems to be efficient only for the two first plants (AERF 2008).

Uses against unwanted algae and plants

2,4-D products are commonly applied to control invasive weeds in several states, such as the Eurasian watermilfoil (*Myriophyllum spicatum*) in the northern tier states and water hyacinth (*Eichhornia crassipes*) in the Gulf Coast states (Helsel, Gerber et al. 1996; Parsons, Hamel et al. 2001).

In Portugal, 2,4-D was successfully used in control trails against two vascular weed species *Myriophyllum aquaticum* and in several trials against *Eichhornia crassipes* (Moreira, Ferreira et al. 1999). These species are among the most invasive weeds in Portugal, predominantly occurring in man-made or man-altered environments.

Carfentrazone-ethyl

General information

Carfentrazone-ethyl is a phenyl triazolinone herbicide that is most commonly used for post-emergent control of terrestrial broadleaf weeds, wheat, barley, rye, oats, corn, soybeans, rice, and for use in turf and ornamental sites.

In 2004, carfentrazone-ethyl was registered by the USEPA for applications in marshes, wetlands, bayous, drainage ditches, canals, streams, rivers, and other slow-moving or quiescent bodies of water (Glomski and Getsinger 2006; Gray, Madsen et al. 2007).

Mode of action

Carfentrazone-ethyl is a contact herbicide that inhibits protoporphyrinogen oxidase (protox inhibitor) in the chlorophyll biosynthesis pathway causing lipid peroxidation and membrane disruption causing plants to become necrotic within hours of treatment (Koschnick, Haller et al. 2004).

Selectivity

Carfentrazone-ethyl is an herbicide with algaecidal activity as well. It is somewhat selective and indicated for the control of various aquatic weeds such as water-hyacinth (*Eichhornia crassipes*), and Salvinia (*Salvinia minima*, *Salvinia molesta*). It is also applied for suppression of watermeals (*Wolffia spp.*), alligatorweed *Alternanthera philoxeroides*, and water primrose (*Ludwigia octovalvis*). It is recommended to be applied to small, active growing weeds (FMC 2004; Glomski and Getsinger 2006; Glomski, Poovey et al. 2006; Gray, Madsen et al. 2007).

Toxicology

The reduced risk nature of this herbicide makes it a good candidate for use near potable water intakes and in environmentally sensitive sites (Glomski, Poovey et al. 2006; Gray, Madsen et al. 2007). Toxicity to aquatic life varies with application rate: rainbow trout is affected at ~1.1 ppm; bluegill at ~1.5 ppm; mysid shrimp at ~1.17 ppm. Surfactants make non-target plant damage more likely. Irrigation must be restricted for 1-14 days after application, depending on crop. There are no post-application swimming or fishing restriction (FMC 2004)

Environmental fate and decomposition

Carfentrazone-ethyl quickly degrades into the chloropropionic acid via pH dependent hydrolysis but the chloropropionic acid has been demonstrated to be just as phytotoxic as carfentrazone. First order half-lives for carfentrazone-ethyl are in the range of 6.5 to 11.1 h in flooded rice fields, and from 3.4 h at pH 9 to 131 h at pH 7 for chloropropionic acid in laboratory hydrolysis studies (Ngim and Crosby 2001). A half-life of 83 h

was reported for a 0.08 ha carfentrazone treated pond (water pH ranging from 6.9 to 9.6) and after 168 h, no residues were detected (Koschnick, Haller et al. 2004). These last authors also found no accumulation of carfentrazone or the chloropropionic acid in sediments.

Formulations and application methods

Carfentrazone-ethyl end-use product registered by the USEPA for aquatic applications is in the market under trade name of Stingray Aquatic Herbicide™. This is a liquid formulation which is emulsifiable in water. It is designed to be mixed with water and applied using a backpack or knapsack sprayer for shoreline applications or a spray boom, handgun or other similar suitable equipment mounted on a boat or vehicle. For control of susceptible submerged weeds in ponds, lakes, reservoirs, and in non-irrigation canals or ditches that have little or no continuous outflow, Stingray can be injected directly into the water through a boat-mounted application system or applied as a subsurface application or as a surface application with a suitable polymer to sink the spray mixture (FMC 2004).

Uses against unwanted algae and plants

Carfentrazone-ethyl is a GUP and has been used for the control of the species indicated above, in the *Selectivity* sub-section.

Copper compounds and derivatives

(See Chapters II and III for more information on copper)

Uses against unwanted algae and plants

Copper is a fast-acting, broad-spectrum, contact herbicide which kills a wide range of aquatic plants and algae. It has long been used in natural and industrial waters for algae control. It is often applied simply as blue copper sulfate crystals. Recently, "chelated copper" has also become popular for aquatic plant and algae management. "Chelate" is a chemistry term meaning combining a metal ion, in this case, copper, with an organic molecule, in this case, triethanolamine or ethylenediamine. Chelated liquid copper products reportedly remain in solution longer than do copper salts (when applied to hard water). Copper that is in solution (suspended in the water) for a longer time has greater effect on the aquatic plants and algae that they are meant to kill.

In situ tests using the commercial algaecide Coptrol examined several methods of applying herbicides to *Undaria pinnatifida*, such as injection into the stipe or midrib, applying a gel formulation, attaching a sponge saturated with active substance to the thallus, and applying compounds inside a bag enclosing the thalli (Sanderson 1996). However results proved to be labor-intensive and had no appreciable impact.

Several brands of copper are available for aquatic plant and algae control, including Cutrine[®], Komeen[®], Copper-Z[®], Nautique[®], Captain[®], Clearigate[®], and K-Tea[®].

Diquat

General information

Diquat, or diquat dibromide, is a fast acting contact herbicide that can be applied to terrestrial, non-crop and aquatic sites. It is a nonselective pesticide and is used to control nuisance submersed and floating aquatic macrophytes.

Mode of action

Diquat is considered a desiccant because it causes a leaf or an entire plant to dry out quickly. It is rapidly absorbed by vegetation, but not readily translocated. It should be applied when target plants are actively growing (Hess 2000).

Selectivity

Diquat provides excellent control of elodea and good method of control for alligatorweed, Eurasian watermilfoil and giant Salvinia (Glomski, Skogerboe et al. 2005).

Tests have shown that diquat is a poor option for the control of seaweeds *Sargassum muticum* (Critchley et al. 1986) and *Undaria pinnatifida* (Sanderson 1996).

Toxicology

Diquat is a moderately toxic chemical. It may be fatal to humans if swallowed, inhaled, or absorbed through the skin. Based on records of suicidal ingestion of diquat by humans and on diquat-feeding studies with

monkeys, it has been concluded that diquat is most harmful to the gastrointestinal tract, kidneys, and liver (Kamrin 1997).

Although absorption is reportedly low following dermal exposure, the demonstrated toxicity of this compound is sufficient to raise serious human health concerns. When absorbed through the skin, some commercial concentrate formulations of diquat can cause symptoms similar to those that occur when it is eaten. Diquat also causes eye irritation. The effects of repeated, or prolonged, dermal contact with diquat range from inflammation of the skin, to systemic poisoning, as evidenced by injury to internal organs, primarily the kidneys. Chronic exposure may damage skin, which allows more absorption of the herbicide (USEPA 1995).

The EPA requires a 14-day interval between treatment of water with diquat and use of treated waters for domestic, livestock, or irrigation purposes. Swimming, fishing and watering of domestic animals should not be allowed for at least 14 days after application of the herbicide to water. The herbicide cannot be used for any purpose in commercial fish processing areas (USEPA 1995).

The USEPA does not consider diquat to cause tumors, teratogenic effects nor fertility problems (USEPA 1995). However, diquat is thought by other researchers to have the potential to cause birth defects. A study showed teratogenic effects in six-day pregnant rats given intravenous injections of diquat. Growth retardation was seen in test animals given extremely high doses of diquat. While no actual teratogenesis occurred in rats given single abdominal injections during the 7th to 14th days of pregnancy, many rats did not have normal weight gain and bone formation in the unborn was decreased (USEPA 1995).

Diquat is slightly toxic to fish. Its toxicity to fish and plankton has been reported in many studies and it appears to be less toxic in hard water. The 8-hour LC₅₀ for diquat in rainbow trout is 12.3 ppm, and 28.5 ppm in Chinook salmon. The 96-hour LC₅₀ in northern pike is 16 ppm and 20.4 ppm in fingerling trout. The shell growth of eastern oysters was not noticeably affected with exposure to 1 ppm of diquat for 96 hours. Some species of fish may be harmed, but not actually killed, by sublethal levels of diquat. Oxygen can become depleted in diquat-treated water by decaying aquatic plants, triggering eutrophication. Research indicates that yellow perch suffer significant respiratory stress when herbicide concentrations in the water are similar to those normally present during aquatic vegetation control programs. There is little or no bioconcentration of diquat dibromide in fish. One investigation into the persistence of diquat in fish showed that one half of the herbicide was lost in less than three weeks (USEPA 1995).

Diquat ranges from moderately toxic to practically nontoxic to birds, depending on the species; acute oral LD₅₀ in twelve young male mallards was 564 mg/kg. Signs of poisoning in these birds included instability, wing-drop and lack of movement. In hens, the oral LD₅₀ for diquat was 200-400 mg/kg. Diquat is not toxic to honey bees but cows are particularly sensitive to the toxic effects of this material (Kamrin 1997). Since diquat is a nonselective herbicide with non-crop use patterns that overlap endangered plant habitats, it may present a danger to nontarget plants, including endangered species.

Due mainly to the risks associated with diquat's dermal toxicity, this product is a poor choice if lower-risk products are effective on the target species. In addition, diquat has low selectivity, which makes it a poor choice if the intention was not to cause a complete extermination of all plants and plankton in the water body.

Environmental fate and decomposition

When diquat comes in contact with soil, it becomes strongly adsorbed to clay particles or organic matter in the soil for long periods of time. The strong chemical bonds formed by diquat adsorption to soil particles make the herbicide biologically and chemically inactive, thus it is unlikely to leach, to be taken up by plants, broken down by microbes in the soil or broken down by sunlight. Field and laboratory tests show that diquat usually remains in the top inch of soil for long periods of time after it is applied and traces of diquat have been found to persist in soil for many years with very little decomposition. However, there is evidence that diquat may eventually saturate all the available adsorption sites on soil clay particles. Groundwater quality can be affected if soil adsorption sites become totally saturated because water moving down through the soil can carry any non-adsorbed herbicide into the groundwater (USEPA 1995; Kamrin 1997). More research is needed for a better understanding of the potential effects on groundwater of long- term, repeated use of diquat.

Formulations and application methods

Diquat-based registered products labeled for control of aquatic weeds include Weedtrine-D and Reward.

Uses against unwanted algae and plants

Diquat has been a particularly valuable tool in aquatic plant management situations when rapid removal of standing vegetation is desired, or when

rapid dispersion via water exchange patterns limits herbicide exposure time to the target submersed species (Glomski, Skogerboe et al. 2005).

In Portugal, diquat has been shown to be successful against invasive vascular weed *Eichhornia crassipes* (Moreira, Ferreira et al. 1999). However, these authors pointed out that due to the inconvenience of a long post-application safety interval (10 days), diquat is considered to be less efficacious than other options such as glyphosate.

Endothall

General information

Endothall is a broad spectrum herbicide and algaecide, applied to aquatic sites as either a dipotassium salt or an N, N-dimethylalkylamine salt.

Mode of action

Endothall acid is a contact herbicide with little translocation that works by interfering with plant respiration, by affecting protein and lipid biosynthesis, and by disrupting plant cell membranes (USEPA 2005).

Selectivity

Endothall has low selectivity and is generally used against submerged aquatic plants such as Eurasian watermilfoil, curly leaf pondweed and hydrilla (Madsen 2006).

Toxicology

Endothall is highly to moderately toxic to mammals, depending on the formulation. Rat oral LD₅₀ is 38 mg kg⁻¹ for technical endothall acid, 182 mg kg⁻¹ for sodium salt, and 206 mg kg⁻¹ for amine salt. Formulated products have lower toxicities: rat oral LD₅₀ is 1,540 mg kg⁻¹ for Hydrothal 191 granular, and 233 mg kg⁻¹ for the liquid formulation. Dermal toxicity is higher than oral toxicity, so precautions must be taken to avoid dermal exposure. Endothall is also an eye irritant, and inhalation of vapors or dusts can cause irritation and injury (EXTOXNET 1995; USEPA 2005).

Endothall is generally nontoxic to fish at concentrations ≤ 500 ppm, but toxicity to fish varies with formulations (e.g. Hydrothol formulations are more toxic to fish than Aquathol formulations). Endothall should be applied when water temperature is 65 °F or above, and plants are actively growing.

Granular formulations are preferred to liquids due to reduced risk of dermal absorption (EXTOXNET 1995; USEPA 2005).

Environmental fate and decomposition

Endothall absorption in aquatic applications is primarily through leaf surfaces. However, some soil post-application activity may occur. Endothall is mobile in water, and breaks down in both soil and water by microbial action at rates which are dependent on temperature and nutrient availability (USEPA 2005).

Formulations and application methods

For aquatic applications, endothall can be applied as a granular or a liquid. Granular applications are made using centrifugal or blower-type spreaders that are mounted to boats. Granular formulations can also be applied to aquatic sites by spreader equipment attached to helicopters. Liquid applications are made using low pressure hand wand sprayers, hand-gun sprayers, or direct metering systems. Aerial application of liquids to aquatic sites is prohibited (USEPA 2005).

Endothall can be found under the following trade names: Aquathol K[®], which is labeled for aquatic weed control in lakes and ponds; Aquatholl Super K[®], which is labeled for aquatic weed control in lakes, ponds, and drainage ditches, and Hydrothol 191[™] and Hydrothol 191 granular[™], both labeled for algae and aquatic weed control in lakes, ponds, and drainage ditches (USEPA 2005).

Uses against unwanted aquatic plants and algae

Endothall is commonly used for the control of nuisance vegetation and algae control in the US, and restrictions vary with state policies. In Washington State for example, Hydrothol 191[™] may only be used at very low concentrations for filamentous algae or blue-green algae control. Lake managers may need to treat several times each season to control algae (Washington State Department of Ecology 2008).

Erioglaucine/ tartrazine (Aquashade[™])

General information

The dyes erioglaucine (Acid Blue 9 or FD&C Blue No. 1) and tartrazine (Acid Yellow 23 or FD&C Yellow No. 5), when combined, act as an aquatic algacide and herbicide that is commonly referred to by the trade name Aquashade™. It can be used in natural or manmade ponds, lakes, fountains, fish farms, and fish hatcheries, but never directly to streams, other natural bodies of water or into any water that will be used for human consumption.

Mode of action

The mixture of erioglaucine and tartrazine controls the wavelength range of the sunlight spectrum required for photosynthesis, thereby inhibiting growth of filamentous algae and submerged aquatic vegetation (USEPA 2005).

Selectivity

Broad-spectrum. The combination of erioglaucine and tartrazine in water bodies is expected to kill all submerged aquatic plants, given the proper dosage and treatment time.

Toxicology

The USEPA concluded that erioglaucine and tartrazine both have very low toxicity potentials. A definitive target organ has not been identified and clinical signs of toxicity were not observed in any study performed using these dyes. Both are rapidly metabolized and excreted in mammals. The product has low acute oral toxicity with no deaths occurring near the limit dose, moderate acute dermal toxicity and provoked no clinical signs of systemic toxicity in the acute oral and dermal studies. Based on the use pattern, the USEPA waived the need for an acute inhalation study. The product was observed to cause slight eye irritation and to be a dermal sensitizer. There was no evidence of neurotoxicity in any study reviewed by USEPA's RED, and no evidence of carcinogenicity was observed in carcinogenicity studies in mice and rats with erioglaucine or tartrazine (USEPA 2005).

A review of aquatic toxicity studies on bluegill fish, rainbow trout, and daphnia studies showed erioglaucine/tartrazine to be "slightly toxic" to aquatic animals and aquatic invertebrates. No risks of concern were observed for any aquatic animal. The dyes are considered to be nontoxic to birds (toxicity studies were performed with bobwhite quail and the mallard duck). Based on USEPA's screening level assessment,

eriolglaucine/tartrazine will have no effect on endangered species of aquatic animals, terrestrial animals, or terrestrial plants (USEPA 2005).

Environmental fate and decomposition

According to USEPA's RED for Aquashade (USEPA 2005) the most likely major route of dissipation of the dyes in an aquatic environment is indirect photolysis, which depends on the nature and concentration of natural photosensitizers as well as on the geographical location and season when the products are used. Biotransformation under anaerobic conditions may also contribute to the dissipation of each dye. The specific chemical nature of photoproducts and metabolites is not known. The dyes are predominantly associated with the water column and have no potential to volatilize from water. Although the dyes are not applied to soils, data indicate they would be unlikely to volatilize from soils. Acid Blue 9 and Acid Yellow 23 do not have the potential to bioaccumulate in fish.

Formulations and application methods

There are four registered end-use products containing the combination of eriolglaucine and tartrazine; each product has a different ratio of the dyes, but in all the product formulations the percent of eriolglaucine is higher than tartrazine. Aquashade may be applied by professional applicators and homeowners by simply pouring the liquid formulation directly into water (Applied Biochemists 1999). Application is recommended early in the growing season while growth is on the bottom of the water body, or later in the season after the killing and/or removal of any existing growth.

Fluridone

General information

Fluridone is a herbicide generally used to control submerged aquatic invasive species. Use sites include freshwater lakes, ponds, reservoirs, and drainage ditches.

Mode of action

Fluridone is a systemic herbicide absorbed slowly by the plant's roots, inhibiting the production of carbohydrate which is the primary source of energy needed for plant growth. Fluridone is considered a "bleaching herbicide," interrupting carotene biosynthesis in newly emerging tissue by

blocking phytoene desaturase (PDS), an enzyme necessary for production of the intermediate pigment, phytofluene. Because phytofluene is not produced, phytoene, another intermediate pigment, accumulates and the carotenoids are not synthesized. Carotenoids are yellow pigments that aid in photosynthesis, and protect chlorophyll pigments from photooxidation under stressful photosynthetic conditions. Damaged chlorophyll limits the photosynthetic process, and plants eventually die (Poovey, GETSINGER et al. 2005).

Selectivity

Most aquatic plant species are susceptible to fluridone, but a few (algae, many floaters, reeds, cattails, and others) are not. It is reported as an excellent control option for *Eurasian watermilfoil* and hydrilla (Poovey, Skogerboe et al. 2004; Poovey, GETSINGER et al. 2005). Although hydrilla has been found to be susceptible to fluridone in the majority of places in the US, it was reported resistant in Florida through a series of plant collections and analyses in water bodies throughout the state. Michel et al. (2004) determined that resistance occurred through mutations at the PDS gene and levels of resistance correlated to independent somatic mutations at this site. Moreover, because resistance factors at the enzymatic (*in vitro*) step matched responses by the plants in the *in vivo* assessments, these investigators suggested that the fluridone-resistant strains may be equally as competitive as the susceptible strains, and may persist as the dominant plant in a given water body even when selection pressure subsides after the dissipation of fluridone (Poovey, Skogerboe et al. 2004).

Toxicology

Fluridone is generally considered to be of low toxicity to mammals. The USEPA classified fluridone in the relatively low toxicity category for acute dermal and eye irritation, and in the very low toxicity category for acute oral, acute inhalation and dermal irritation. It is not a dermal sensitizer. The USEPA found no indication of reproductive or neurotoxicant effects from fluridone and classified it as “not likely to be carcinogenic to humans” (USEPA 2004).

Fluridone is slightly to moderately toxic to aquatic organisms. There are no restrictions on use of treated water, except for irrigation of turf, forage, and food crops (all 30 days). N-methyl Formamide (NMF) is the major degradate when fluridone is applied to water bodies. A limited number of studies have been conducted under field conditions and these studies suggest that NMF is undetectable in water bodies treated with fluridone at the maximum

application rate. The toxicology database for NMF is limited to one developmental study that was reported in the literature. NMF is not a metabolite in foods (USEPA 2004).

Environmental fate and decomposition

Fluridone strongly absorbs to organic matter in soil and in water, and displays extremely limited soil-activity or soil-mobility. In water, fluridone decomposition is triggered primarily by light; however some breakdown occurs by microorganisms and aquatic plants. In soil, fluridone is primarily broken down by microbial activity. Persistence ranges from 21 days in water to 90 days in hydro-soils, depending upon light, temperature and microbial populations (Koschnick, Haller et al. 2003; Poovey, Skogerboe et al. 2004).

Formulations and application methods

Liquid and granular. Currently, two slow release pellet formulations of fluridone are commercially available, Sonar SRP and Avast SRP4. These are clay-based formulation containing 5% active ingredient. Sonar SRP was reported to have a release rate of 10 to 16 days depending on the amount of agitation subjected to the pellets (Mossler, Shilling et al. 1993). Applications can be done before or during active growth period for target weeds, as long as they are visible and water movement is minimal.

Uses against unwanted algae and plants

Low-dose applications (10 to 15 $\mu\text{g ai L}^{-1}$) of fluridone have been the management tool of choice to control the spread of hydrilla and restore open water and native vegetation on large water bodies in the US. The widespread and frequent use of this herbicide is primarily due to the excellent cost-effective efficacy on hydrilla, and the low toxicological risk that fluridone poses to the non-target aquatic community and to human health (Poovey, GETSINGER et al. 2005).

Fluridone has also been used against Eurasian watermilfoil. In Michigan for instance, small lakes that were dominated by Eurasian watermilfoil over more than 50% of their littoral regions were treated with low doses of fluridone. This treatment confirmed that fluridone treatments can control Eurasian watermilfoil and hydrilla without impacting most native species if fluridone concentrations were initially at least 5 $\mu\text{g/L ai}$ and maintained above at least 2 $\mu\text{g/L ai}$ for at least 60 days (Madsen and Getsinger 2002).

Glyphosate

General information

Glyphosate is a broad spectrum herbicide used for control of terrestrial and aquatic invasive species and is among the most widely used pesticides by volume in the US. Prevalent use sites include hay pasture, soybeans and field corn (USEPA 1993).

Mode of action

Glyphosate is a systemic herbicide that blocks the production of certain proteins needed for plant growth when absorbed through the leaves. Its mode of action is unique in that it is the only molecule that is highly effective at inhibiting the enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) of the shikimate pathway (i.e. the biosynthetic sequence employed by plants and bacteria such as *E. coli* to generate the aromatic amino acids phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp)). How glyphosate-induced inhibition of the shikimate pathway actually kills plants is not entirely clear, but it appears to inhibit the aromatic amino acid biosynthesis pathway and may inhibit or repress chlorismate mutase and/or prephenate hydratase (USEPA 1993; Duke and Powles 2008).

Selectivity

In aquatic applications, glyphosate is effective on emerged plants and shoreline vegetation. Glyphosate has been successful in the control of various aquatic weeds including alligatorweed, giant Salvinia (Madsen 2006), waterhyacinth (Moreira, Ferreira et al. 1999), and common reed (*Phragmites australis*) (Farnsworth and Meyerson 1999).

In contrast, submerged aquatic plants are either resistant or affected only by very high glyphosate concentrations. No adverse effects on the growth of elodea (*Elodea canadensis*), water milfoil (*Myriophyllum spicatum*), and wild celery (*Valisneria americana*) were found with glyphosate concentrations of up to 1 mg L⁻¹ (Kerr 2009 and references therein).

A large number of studies with a variety of green algae, blue-green algae, diatoms, and periphyton indicate that glyphosate is slightly toxic to practically non-toxic to most algae. Most algae tolerate concentrations of glyphosate greater than 1 mg L⁻¹. Species of algae vary in their sensitivity to glyphosate in terms of population growth (Giesy 2000). Few data are available on effects to marine algae, as most toxicity tests have been performed on freshwater species (Kerr 2009).

Toxicology

Glyphosate has been found by the USEPA to be of low toxicity to humans and to other mammals, no more than slightly toxic to birds, practically non-toxic to aquatic invertebrates, honeybees and fish (USEPA 1993).

Glyphosate toxicity varies depending on the inert ingredients (e.g. surfactants, solvents and adjuvants) used in the end-products. For instance, end-use product Roundup is reported as significantly more toxic to trout and bluegill than end-use products Accord and Rodeo (both registered for application in fresh and brackish water)(Kamrin 1997; Dow AgroSciences 2008; Duke and Powles 2008). Roundup includes the terrestrial surfactant polyethoxylated tallowamine (POEA) and is not registered for aquatic applications.

Mann and Bidwell (1999) found that LC₅₀ values for 48-h exposure of four species of Australian tadpoles (*Crinia insignifera*, *Heleioporus eyrei*, *Limnodynastes dorsalis*, and *Litoria moorei*) to glyphosate, in laboratory settings, ranged from 3.9 to 15.5 mg AI L⁻¹ for Roundup (glyphosate plus POEA surfactant); 108 to 161 mg AI L⁻¹ for technical grade glyphosate acid, and 450 mg AI L⁻¹ for glyphosate isopropylamine salt (the latter two formulations lack the POEA surfactant). Perkins et al. (2000) found LC₅₀ values for 96-h exposure of *Xenopus laevis* tadpoles, also in laboratory settings, of 12.4 mg AI L⁻¹ for Roundup, 6.8 mg/L for the POEA surfactant alone, and 9,729 mg AI L⁻¹ for Rodeo (aquatic form of glyphosate that lacks the POEA surfactant). The results from these studies suggest that the high mortality associated with commercial forms of Roundup is actually due to the POEA surfactant and not to glyphosate itself.

Glyphosate has been determined to be practically non-toxic to mammals by ingestion with an acute oral LD₅₀ of 5,600 mg kg⁻¹ b.w. in rats. NOEL for chronic toxicity to rats at 362 mg kg⁻¹ b.w. per day was 8,000 ppm and LOEL at 940 mg kg⁻¹ b.w. per day was 20,000 ppm. The reported acute LD₅₀ values for dermal effects ranged from >5,000 to 7,940 mg kg⁻¹ for rabbits. Subchronic oral toxicity studies of glyphosate with rats and dogs indicate that oral doses of up to 2,000 ppm do not significantly affect behavior, survival, or body weight. Laboratory studies of the chronic effects of glyphosate show that it is slightly to practically non-irritating to rabbits' eyes. No significant reproductive, teratogenic, mutagenic, or carcinogenic effects from exposure to concentrations of up to 300 ppm were reported in 20-year laboratory studies with rats, dogs, rabbits, and mice (USEPA 1993).

Giesy *et al.* (2000) reviewed the data available on glyphosate toxicity (with and without POEA surfactant) to microorganisms, and found that acute toxicity EC₅₀ values ranged from 2.1 to 189 mg/L. EC₅₀ values for tests with

microorganisms using the glyphosate isopropylamine salt ranged from 72.9 to 412 mg L⁻¹, and NOEC values ranged from 7.9 to 26.5 mg L⁻¹ (Giesy, Dobson et al. 2000). These NOEC values are well above expected treatment residual concentrations thus, impacts to non-target microorganisms are not likely. Impacts in estuarine conditions with high concentrations of suspended sediment, which interfere with glyphosate activity, would be even less likely (Kerr 2009).

Glyphosate is practically non-toxic to bobwhite quail on the basis of acute oral toxicity: LD₅₀ > 2,000 mg kg⁻¹ for bobwhite quail given a single oral dose of technical glyphosate. The 8-day dietary LC₅₀ of the chemical is > 4,000 ppm for both mallard ducks and bobwhite quail. These data indicate that the chemical is slightly toxic to birds. Avian reproduction studies indicate reproductive impairment would not be expected at a dietary level of up to 1,000 ppm (USEPA 1993). Little or no data are available on toxicity of surfactants to birds.

A 48-hour LC₅₀ of 780 ppm was found for *Daphnia magna* exposed to technical glyphosate. A fish lifecycle study indicates technical glyphosate has a MATC greater than 25.7 ppm. No effect was observed at the highest level tested. A *Daphnia magna* life cycle study with an MATC of >50 - <96 ppm reported reduced reproductive capacity, the most sensitive parameter. A glyphosate formulation (containing surfactants) was tested several times with different invertebrates. The LC₅₀ values ranged from 3 mg L⁻¹ for *Daphnia* to 62 mg L⁻¹ for *Gammarus* indicating a moderately toxic material for *Daphnia* and no more than slightly toxic for *Gammarus* (USEPA 1993).

Some studies were performed on marine and estuarine species. A 96-hour LC₅₀ of 281 ppm was determined for grass shrimp (*Palaemonetes vulgaris*). In a study on fiddler crabs (*Uca pugilator*), it was determined that the 96-hour LC₅₀ is 934 ppm glyphosate. Both of these studies indicate technical glyphosate is practically non-toxic to grass shrimp and fiddler crabs. An embryo-larvae 48-hour TL₅₀ for Atlantic oyster greater than 10 ppm indicating glyphosate is slightly toxic (USEPA 1993).

A 3-year study assessed short- and long-term fate, plus potential effects to marine biota associated with repeated applications of Rodeo to control smooth cordgrass (*Spartina* spp.) in estuarine Willapa Bay, WA (Kilbride and Paveglio 2001). Plots were established at three intertidal locations on exposed mudflats and along the edge of a *Spartina* meadow. The plots were hand sprayed with 5% Rodeo solution combined with 2% LI-700, a nonionic surfactant. The concentration of glyphosate in the sediment from mudflat plots declined by 88- 96% from the concentration measured 1 day after the application to the one observed 1 year after a second round of Rodeo application. In contrast, glyphosate concentrations in *Spartina* plots increased 231% to 591% from the first through the third year. The authors

suggested that this increase was probably because *Spartina* rhizomes did not readily metabolize or exude the glyphosate. The authors also compared glyphosate concentrations from mudflat and *Spartina* plots with toxicity test values for two marine biota indicators (i.e. the Pacific oyster, *Crassostrea gigas*, and an estuarine amphipod, *Eohaustorius estuaries*) and concluded that that even under worst-case conditions for these types of applications and ecosystem, short- and long-term detrimental effects to aquatic biota from repeated application of Rodeo for *Spartina* control are negligible.

Variation in the aquatic toxicity of glyphosate has been attributed to the dilution water, temperature, formulation, and the amount of suspended sediment in the water. Toxicity appears to increase with temperature, and decrease with elevated pH and suspended sediment (Kerr 2009).

Environmental fate and decomposition

Glyphosate is degraded by microbial activity with a moderate half-life of about 60 days. Glyphosate is strongly absorbed by soil particles and organic matter with very little movement to soil and groundwater, although in soils with macropores and pronounced preferential flow, glyphosate could move readily to groundwater. The major glyphosate decomposition product, aminophosphonic acid (AMPA), is significantly more mobile than glyphosate in soil. Glyphosate is not volatile, so atmospheric contamination is not expected to occur (USEPA 1993).

Experiments carried out at Mai Po Nature Reserve (Hong Kong) assessed glyphosate's environmental fate and impacts to non-target fish onto that wetland system (Tsui and Chu 2008). These authors applied Roundup with a hand-held sprayer to an estuarine and a freshwater pond. Surface water and sediment were sampled routinely for glyphosate concentrations following one month of application. Concurrent *in situ* bioassays using native fish species (i.e., fish fry of grey mullets, *Mugil cephalus*, in the estuarine pond, and fry of freshwater mud carp, *Cirrhinus molitorellai*, in the freshwater pond) were performed. One day after the application, up to 52% of the glyphosate measured in the surface water had been transported to the unapplied regions by wind-driven current in the estuarine pond. In both ponds, glyphosate concentrations in the water column decreased rapidly after 1–3 days post treatment, and continued to decrease over time. The authors observed that the persistence of glyphosate in the freshwater pond was longer than in the estuarine system, and suggested that this was likely due to higher concentrations of chelating metals (i.e. Cu and Fe) in the freshwater pond sediments, which could have reduced the bioavailability of glyphosate to microbial decomposers. The authors also registered that the fish used in the bioassays (both in applied and unapplied areas) showed

similar survival rates, indicating that the use of Roundup at the provided application rate posed no obvious short-term effects on native fish species.

Formulations and application methods

Rodeo, Accord (both produced by Dow AgroSciences), AquaPro (produced by SePro) and Aquamaster (produced by Monsanto) are some of the trade names of glyphosate-containing herbicides currently registered for applications to aquatic sites, including estuaries. Aquatic treatments with glyphosate should be done when the target vegetation is actively growing. These herbicides are usually mixed with a surfactant and applied using hand held sprayers or low ground pressure amphibious vehicles.

Uses against unwanted algae and plants

Glyphosate is commonly used in the control of various freshwater weeds and is one of the few active ingredients registered for applications in brackish waters, including estuaries.

Glyphosate has been used against smooth cordgrass (*Spartina alterniflora*) in Washington estuaries (Simenstad, Cordell et al. 1996; Kilbride and Paveglio 2001; Major III, Grue et al. 2003) and in the San Francisco Bay estuary (Olofson 2008) with different levels of success, depending mostly on application rates and associated techniques such as the use of surfactants (Patten 2002; Roberts and Pullin 2008).

Glyphosate was found to be a poor control method for *Spartina anglica* growing in Northern Ireland estuaries (Hammond and Cooper 2002).

Controlled experiments showed that glyphosate was also inefficient at controlling gametophytes of two kelp species, *Undaria pinnatifida* and *Ecklonia radiata* (Burrige and Gorski 1998).

In Hawaii, efforts to eliminate invasive red mangrove (*Rhizophora mangle*) from the Wai 'Opae Marine Life Conservation District in Puna, Hawai'i Island, included the use of glyphosate (Kobsa, Messick et al. 2008; Kobsa, Messick et al. 2009). The project, led by the Malama O Puna, a local non-profit corporation which focuses on the environment, consisted of controlled applications of glyphosate (Aquamaster™) and imazapyr (Habitat™) in both foliar applications and by injection into trees' trunks. Foliar applications of glyphosate at 0.5, 1, 4 and 6% and using a surfactant (mse 0.5%) were not effective, as propagules released from dying trees were 100% viable (in contrast with the imazapyr foliar treatment, which resulted in only 6% viable

propagules). The injection trials were successful for both herbicides. It consisted of drilling 1-2 holes per tree trunk and introducing glyphosate (0.6-1 mL per inch of trunk diameter) or imazapyr (0.1-0.4 mL per inch of trunk diameter) into the holes. A total of 9,127 mangrove trees on nearly 7 acres were subjected to the treatment, using the total amount of 16.5 liters of Aquamaster™ herbicide.

Imazapyr

General information

Imazapyr is a systemic and relatively selective herbicide, usually applied for the control of broad leaf, brush and floating leaf aquatic weeds. Imazapyr is the only USEPA-registered pesticide that is approved for applications in marine sites. It is also approved for use in estuarine sites. Applications of imazapyr can only be carried out by federal or state agencies and must be supervised by individuals who are certified as aquatic pest control applicators and authorized by state or local governments. Applications of imazapyr to control invasive species that are not listed in the label are limited to species that have been classified as nuisance by a federal or state government entity (BASF 2004)

Mode of action

Imazapyr interferes with vegetal metabolism by preventing the synthesis of branched-chain amino acids (i.e. aliphatic amino acids valine, leucine and isoleucine) and gradually starve the plant (animals do not produce these amino acids but rather acquire by consuming plants)(USEPA 2006; Olofson 2008).

Selectivity

Imazapyr is efficient at controlling ditch bank and emergent weeds such as parrotfeather, and is an excellent control option for alligatorweed, and waterhyacinth, but poor for the control of giant Salvinia. Imazapyr can be used where total vegetation control is desired or in spot applications (BASF 2004; USEPA 2006). Other aquatic algae appear to be substantially less sensitive. The most sensitive species of algae tested was a unicellular green algae () with an EC50 of about 0.2 mg L⁻¹ for growth. Some algal species appear to be stimulated rather than inhibited by imazapyr concentrations of up to 100 mg L⁻¹ (Kerr 2009).

Some species of plants, including aquatic plants, may develop resistance to imazapyr. Bioassays conducted on *Chlorella emersonii* indicated that resistant strains may be less sensitive by a factor of 10 (Kerr 2009).

Toxicology

The USEPA determined that dietary risks (food and drinking water), residential and commercial handler dermal and inhalation risks and post-application exposures, as well as aggregate risk for human health, are below the agency's level of concern. However, the agency determined that imazapyr is a primary eye irritant (Toxicity Category I) (USEPA 2006).

The USEPA classified imazapyr as "practically non-toxic" to wildlife, including mammals, birds, fish, and aquatic invertebrates, but alerted to the fact that there are ecological risks of concern associated with the use of imazapyr that could not be precluded. Specifically, the agency pointed out that potential risks for non-target terrestrial plants, aquatic vascular plants, and federally listed threatened and listed endangered species which include aquatic vascular plants, terrestrial and semi-aquatic monocots and dicots (USEPA 2006).

The USEPA considers imazapyr as practically non-toxic to mammals via oral or dermal administration based on acute and chronic studies conducted with a variety of mammalian species. The reported acute oral LD50 for technical imazapyr in rats is greater than 5,000 mg kg⁻¹ body weight. No observable effect was noted for any formulation of imazapyr administered dermally. Very few inhalatory studies were performed and none tested concentrations high enough to determine acute toxicity. Inhalatory effects at sublethal concentrations (<5 mg L⁻¹ aerosol) were found with technical grade imazapyr resulting in slight nasal discharge and congested lungs. Technical grade imazapyr and imazapyr isopropylamine salt were both found to be moderately irritating to rabbit eyes with complete recovery within 7 days. Technical grade imazapyr is reported as mildly irritating to rabbit skin. Commercial formulations of imazapyr appear to be less toxic via dermal exposure. Chronic and subchronic toxicity studies with imazapyr with dogs, mice, and rats did not suggest any systemic toxic or carcinogenic effects (USEPA 2006).

Few toxicity studies have examined the effects of imazapyr on birds. No adverse effects were noted at imazapyr concentrations of up to 5,000 ppm in the diet of quails. Based on the highest doses tested and the USEPA ecotoxicity categories, these results suggest that imazapyr is moderately or less toxic orally to birds (USEPA 2006). No data exist for the potential toxicity of imazapyr to shorebirds. No studies exist on toxicity to raptors or on preening or inhalation exposure potentials (Kerr 2009).

Imazapyr has been classified as having low toxicity to aquatic invertebrates. 48-hour exposure of *Daphnia magna* to a 50% imazapyr formulation resulted in an EC₅₀ of 373 mg imazapyr a.e. L⁻¹. In another study, 48-hour exposure of *Daphnia* to Arsenal® (identical to Habitat®) with an unspecified surfactant resulted in a LC₅₀ of 350 mg Arsenal L⁻¹ (79.1 mg imazapyr a.e. L⁻¹) and a NOEC of 180 mg Arsenal L⁻¹ (40.7 mg imazapyr a.e. L⁻¹). Other studies also reported 24 and 48-hour LC₅₀ concentrations of greater than 100 mg L⁻¹, the highest dose tested (“HDT”), in static tests conducted with newly-hatched *Daphnia*. Chronic studies reported no adverse effects on survival, reproduction or growth of 1st generation of *Daphnia* after 7, 14 and 21-days of exposure at concentrations up to 97.1 mg L⁻¹, the HDT (Kerr 2009).

Testing with other invertebrate species that exhibit alternative life cycles has been limited to survival of pink shrimp (*Penaeus duorarum*) and growth studies with the Eastern oyster (*Crassostrea virginica*). Acute toxicity to pink shrimp was determined at LC₅₀ >132 mg imazapyr a.e. L⁻¹, the HDT, which was also the NOEC. The EC₅₀ for growth inhibition of the Eastern oyster was established at a concentration greater than 132 mg imazapyr a.e. L⁻¹, with the NOEC set at this concentration, the HDT. A recent microcosm study (Fowlkes, Michael et al. 2003) analyzed benthic macroinvertebrates in a logged pond and confirmed the low toxicity of imazapyr to benthic freshwater macroinvertebrates. The study analyzed macroinvertebrate community composition, chironomid deformity rate, and chironomid biomass and concluded that imazapyr did not affect the macroinvertebrate community at the concentrations tested. The NOEC was determined to be greater than 18.4 mg L⁻¹.

A number of standard bioassays were submitted to the USEPA in support of the registration of imazapyr and indicated very low toxicity to fish with 96-hr LC₅₀ values greater than 100 mg L⁻¹ in most studies (USEPA 2006). One study registered a LC₅₀ for juvenile rainbow trout 22,305 mg imazapyr a.e. L⁻¹. The formulation used in this test was Arsenal®, a terrestrial formulation identical to Habitat® that did not contain any surfactants (King, Curran et al. 2004).

Environmental fate and decomposition

Research indicates that imazapyr has low potential to bioaccumulate and exhibits rapid rate of decay in both water and sediment after application to estuary mud. It is primarily broken down in water by photolysis (half-life of 2.5-5.3 days) but sunlight reduced imazapyr below detection quickly in estuary water (avg. 40 hrs). The active ingredient disappeared from mudflat sediment within average of 400 hrs (Patten 2003).

Imazapyr is active in small quantities, but may take several weeks to act. It can be mobile within roots and transferred between intertwined root systems (root grafts) of many different plants and/or to several species. Movement of imazapyr via root grafts or by exudates (which is a defense mechanism of those plants) may therefore adversely affect the surrounding vegetation. This movement of herbicide may be compounded when imazapyr is incorrectly over applied. Movement of soil particles that contains imazapyr can also potentially cause unintended damage to desirable species (Patten 2002; USEPA 2006).

Formulations and application methods

Imazapyr is registered by the EPA for aquatic herbicidal applications under the trade name Habitat®. Imazapyr is applied either as an acid or as the isopropylamine salt. Aquatic applications of imazapyr can be made as a liquid. Application methods include aerial and application to water via boat. Aqueous imazapyr formulations may be mixed with surfactants or oils for application. Applications to smaller areas may be made with handheld equipment, including backpack sprayers, sprinkling cans, and handgun sprayers (USEPA 2006).

The addition of surfactants (e.g. lecithin-based, Liberate, or methylated vegetable oil, Competitor) is recommended to lower surface tension of the liquid formulation thus improving the spreading over the leaves, adherence to the plant, and penetration of the leaf cuticle (Pless 2005; Olofson 2008).

Uses against unwanted algae and plants

Research on alternative herbicides for the control of *Spartina alterniflora* in Washington state estuaries has shown that, when applied at 6 to 12 L ha⁻¹, at the rate of 19 to 38 L per ha spray volume, imazapyr is excellent for the control of this invasive species and could provide cost effective control at approximately \$600 per ha over large treatment areas (> 20 to 100 ha per day) (Patten 2002; Hedge, Kriwoken et al. 2003). Imazapyr has also been used in *Spartina* control programs in the San Francisco Bay Estuary (Pless 2005; Olofson 2008).

A recent study (Mozdzer, Hutto et al. 2008) compared the efficacy of imazapyr (Habitat™) to that of glyphosate (Rodeo™) on 1-ha common reed (*Phragmites australis*) monoculture in a shallow borrow pit at Kiptopeke State Park, Virginia. Six foliar experimental treatments were applied consisting of variable concentrations and application seasons for glyphosate (2%) and imazapyr (2 and 5%). Experimental plots were monitored yearly for two years after treatment. These authors found that

foliar applications of imazapyr are statistically superior to glyphosate in reducing this invasive wetland angiosperm species. They found no significant differences between the 2 and 5% formulations. Both herbicides were more effective in reducing *P. australis* if applied early in the growing season. No significant differences in plant recolonization by non-*Phragmites* were observed between herbicide treatments over the two-year period.

In Hawaii, efforts to eliminate invasive red mangrove (*Rhizophora mangle*) from the Wai 'Opae Marine Life Conservation District in Puna, Hawai'i Island, included the use of imazapyr (Kobsa, Messick et al. 2008; Kobsa, Messick et al. 2009). The project, led by the Malama O Puna, a local non-profit corporation which focuses on the environment, consisted of controlled applications of glyphosate (Aquamaster) and imazapyr (Habitat) in both foliar applications and by injection into trees' trunks. Injection treatment consisted of drilling 1-2 holes per tree trunk and introducing glyphosate (0.6-1 mL per inch of trunk diameter) or imazapyr (0.1-0.4 mL per inch of trunk diameter) into the holes. Foliar applications of imazapyr at 0.5 and 1% using a surfactant (mse 0.5%) were highly successful with only 6% of propagules released from dying trees being viable (in contrast with the glyphosate foliar treatment, which resulted in 100% viable propagules). The injection trials were successful for both herbicides.

Penoxsulan

General information

Penoxsulan is a systemic herbicide used to control broadleaf weeds. Common use sites include golf courses, sports fields, sod farms, commercial and residential lawn care. Penoxsulam is also used against broadleaf, sedge, and grass weeds in transplanted, dryseeded, and water-seeded rice (USEPA 2004).

Penoxsulam was recently approved by the EPA (in July of 2007) for the management of freshwater aquatic vegetation in ponds; lakes; reservoirs; marshes; wetlands; bayous; drainage ditches; non-irrigation canals; and slow-moving or quiescent bodies of water; including shoreline and riparian areas within or adjacent to these and other aquatic sites (USEPA 2004; SePRO 2009).

Mode of action

Penoxsulam disrupts the internal growth processes of weeds by inhibiting acetolactate synthase (ALS). As an ALS inhibitor, penoxsulam targets an

enzyme found only in plants and microorganisms and is therefore not expected to pose a threat to wildlife or humans. Death of susceptible weeds occurs in 2 to 4 weeks following application (USEPA 2004).

Selectivity

Penoxulam is effective in selective control of emergent, floating and submersed aquatic weeds like hydrilla, Eurasian watermilfoil, water hyacinth, water lettuce, Salvinia and duckweed.

Toxicology

Penoxsulam is highly toxic to aquatic organisms on an acute (single, high dose) basis, but practically nontoxic to birds on an acute basis and slightly toxic to birds on a dietary basis; human health risks are considered negligible (USEPA 2004).

Environmental fate and decomposition

A study characterized the aqueous photodegradation of penoxsulam using ¹⁴C-labeled isotopes of penoxsulam in a “merry-go-round” reactor (Jabusch and Tjeerdema 2006). The results suggest that penoxulam decomposes within 2-3 days into at least seven photodegradation products. Of these seven photoproducts, four were identified and found to decompose within 15 days. The authors detected no trace of penoxulam and its photoproducts in rice fields 6 weeks after treatment, but recommended the uncharacterized risk of photodegradation products of penoxsulam to aquatic plant and microbial communities to be more fully addressed.

The potential for penoxsulam to accumulate in the food chain (bioconcentrate) is considered to be low. Penoxsulam is easily adsorbed by soil and has low to moderate leaching potential in most soil types. In soil, penoxsulam is believed to be broken down by microbial decomposition (USEPA 2004).

Formulations and application methods

Penoxsulam's registered end-use product for aquatic weed control in the U.S. is sold by SePRO under the trade name Galleon™ herbicide (liquid and granular). The liquid formulation can be sprayed onto vegetation or

applied directly into water. Foliar applications require the use of a surfactant.

Uses against unwanted algae and plants

No specific information available (see *Selectivity* subsection above).

Triclopyr salt (TEA)

General information

Triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) is a systemic pyridinecarboxylic acid compound that generally provides selective control of broadleaf plants with little injury to most grass species. This herbicide is used in the control of broadleaf weeds and woody plants on rights-of-way, rangeland and pastures, forests, lawns, industrial sites, and other non-crop areas. It is also used for broadleaf weed control in rice production (USEPA 1998).

In 2003, the EPA registered the triethylamine salt formulation of triclopyr (TEA) for the control of immersed, submersed and floating aquatic plants in aquatic sites such as ponds, lakes, reservoirs, non-irrigation canals, seasonal irrigation waters and ditches which have little or no continuous outflow, marshes, and wetlands, including broadleaf and woody vegetation on banks and shores within or adjacent to these and other aquatic sites but not salt water bays or estuaries (SePRO 2009).

Mode of action

Triclopyr acts as a synthetic auxin, giving a plant an auxin overdose 1,000 times natural levels, which disrupts the hormonal balance and interferes with growth. The effects occur at the cellular level first, then exterior effects are seen. Ethylene and protein production in the plant increases first then after about one week, epinasty, abnormal leaf formation and stem swelling occur. Sometimes plants may resprout and then die. Low concentrations of triclopyr can stimulate RNA, DNA, and protein synthesis leading to uncontrolled cell division and growth, and, ultimately, vascular tissue destruction. Conversely, high concentrations of triclopyr can inhibit cell division and growth (Swadener 1993).

Selectivity

Triclopyr is successful in the control of invasive aquatic and wetland weeds such as Eurasian watermilfoil (*Myriophyllum spicatum*), purple loosestrife (*Lythrum salicaria*), waterhyacinth (*Eichhornia crassipes*), alligatorweed (*Alternanthera philoxeroides*), American frogbit (*Limnobium spongia*) and wild taro (*Colocasia esculenta*), among others.

Toxicology

Triclopyr is low in toxicity via the oral route, it is a non-irritating to the skin of rabbits but dermal sensitization occurs if applied to the skin of guinea pigs. Inhaled triclopyr is low in toxicity to rats. No reports of systemic poisoning resulting from ingestion of triclopyr were found. There is no evidence that triclopyr causes unscheduled DNA synthesis or acts as a mutagen. In feeding studies with mice and rats, no compound-related tumors are observed in male animals. However, there is a significant increase in the presence of mammary gland adenocarcinomas in female mice and rats fed triclopyr at 36 mg/kg/day for 2 years. The Carcinogenicity Peer Review Committee (CPRC) at the USEPA classified triclopyr as “not classifiable as to human carcinogenicity”(USEPA 1998).

Both forms of triclopyr are slightly to practically non-toxic to birds such as quail (for BEE, LD50 = 735 to 849 mg/kg), mallard ducks (for TEA, LD50 = 2055 mg/kg), and zebra finch (for BEE, LD50 = 1,627 to 2,277 mg/kg). Triclopyr TEA is practically non-toxic (LC50 > 100 ppm) to bluegill sunfish (*L. macrochirus*), rainbow trout (*O. mykiss*), and fathead minnow (*P. promelas*) in acute studies. The major metabolite, TCP, is moderately toxic to fish (LC50 >1 to 12) including several species of salmon and the previously mentioned fish species. The elimination half-life in crayfish (*P. clarkii*) is 7 to 17 days. Triclopyr TEA is practically non-toxic to waterflea (*Daphnia magna*) with an LC₅₀ of 1,496 mg L⁻¹. Levels of triclopyr TEA that elicit reproductive impairment in the waterflea under laboratory conditions are not expected in freshwater systems with current labeled rates (TEA concentrations greater than 80.7 mg L⁻¹)(USEPA 1998).

Environmental fate and decomposition

Upon application to an aquatic system, triclopyr TEA dissociates to triclopyr acid, which subsequently degrades to the primary metabolite, 3,5,6-trichloropyridinol, or TCP. In addition, 3,5,6-trichloro-2-methoxypyridine, or TMP, is a common metabolic degradate. It is uncertain whether TMP is a direct product of triclopyr, TCP, or both. Evidence from laboratory investigations suggests that triclopyr will degrade in natural waters primarily

due to the presence of direct sunlight, through the process of photolysis, producing oxamic and other non-halogenated, low molecular weight organic acids as the photoproducts. These results also indicate that in the absence of light, such as conditions of murky natural water, direct shading, or floating vegetation mats, the decomposition of triclopyr by microbial action would be quite slow, producing the metabolites TCP and TMP only after several months. In addition, chemical hydrolysis would not be a major route of triclopyr decomposition. However, the evidence from field studies examining triclopyr dissipation in natural waters would seem contradictory to the conclusions which might be drawn from laboratory studies. Field studies indicate that triclopyr in natural waters degrades rather quickly, but at least partially independent of the action of direct photolysis. Applications of triclopyr to the surface of the water, or subsurface below dense plant mats yielded similar dissipation half-lives, with TCP and TMP being the major decomposition products (Petty, Getsinger et al. 2003).

Field studies clearly show that triclopyr and TCP are found in fish and shellfish tissues in direct proportion to the residues found in water, and deplete from these organisms at rates roughly equivalent to the dissipation from the water column (typical fish half-lives of 5 to 8 days), indicating a low bioconcentration potential. The TMP metabolite appears to bioaccumulate in certain fish tissues, especially those higher in fat, such as the inedible and visceral tissues. However, this is short-lived, with clearance from fish tissue mirroring the decline of TMP in the water column. Fish half-life values for TMP ranged from 4.6 to 11.6 days. This environmentally compatible degradation scenario, combined with its excellent toxicological profile, and ability to selectively control exotic weedy species, will make triclopyr a valuable tool for restoring and managing aquatic ecosystems (Petty, Getsinger et al. 2003).

Formulations and application methods

Triclopyr is registered for aquatic use under trade names Renovate 3, Garlon 3A, Garlon 4 among others. Foliar applications using surface or aerial equipment are recommended for the control of floating and emerged weeds and woody plants with triclopyr. Repeated applications may be necessary and use of a non-ionic surfactant in the spray mixture is recommended to improve control. Submerged weeds other susceptible submerged weeds in ponds, lakes, reservoirs, etc should be treated with either surface or subsurface applications through boat-mounted distribution systems. It is recommended that when treating target plants that are 6 feet

below the surface of the water, trailing hoses are to be used along with an aquatic approved sinking agent (SePRO 2009).

Uses against unwanted algae and plants

Triclopyr can effectively control the weed species listed in the *Selectivity* subsection, causing little or no harm to the native aquatic plants, such as cattails, rushes, reeds, grasses, and submerged monocots (Netherland and Getsinger 1992; Petty, Getsinger et al. 2003; Poovey, Getsinger et al. 2004; SePRO 2009).

Applications 20% triclopyr ester in oil to basal bark of invasive red mangrove (*Rhizophora mangle*) not in standing water have also been reported (Motooka, Castro et al. 2003).

USEPA-registered Aquatic Algaecides, Bactericides and Viruscides

Algaestats (Hydrogen peroxide)

General information

Algaestats are EPA-registered sodium carbonate peroxyhydrate products that perform as fast acting algaecides in the control blue-green algae. When applied to water, these products break down into sodium carbonate and hydrogen peroxide (H₂O₂). Hydrogen peroxide is the actual active ingredient. The USEPA first registered sodium carbonate peroxyhydrate products in 2002 as a biopesticide for use as an algaecide and fungicide on ornamental plants and turfs. Later in 2004, the USEPA registered sodium carbonate peroxyhydrate for use in ponds, lakes, reservoirs, and drinking water sources (USEPA 2002).

Algaestats do not kill algae outright but instead inhibit their growth, preventing bloom formation. Managers can use these products as an alternative to copper-based algaecides and apply algaestats to the water to prevent algal blooms or to treat existing filamentous algae (USEPA 2002).

Mode of action

Hydrogen peroxide appears to have both external and internal modes of action upon contact with algal cells depending upon their sensitivity and

H₂O₂ concentration. It reacts in contact with organic materials (algae, detritus, etc), rapidly forming water molecules and releasing dissolved O₂ into the water column (Quimby, Kay et al. 1988; ICF International 2006).

Selectivity

The efficiency of peroxide as a biocide on algae and other related aquatic plant life has been successfully demonstrated (Kay, Quinley et al. 1984; Gregg, Rigby et al. 2009). Cysts of *Alexandrium catenella* and *Alexandrium tamarense* were fatally damaged at hydrogen peroxide concentrations of 30 mg L⁻¹ after 48 h exposure and all cysts were destroyed (no germination occurred) after exposure to 100 mg L⁻¹ for 96 h (Ichikawa, Wakao et al. 1992; Montani, Meksumpun et al. 1995). Bolch and Hallegraeff (1993) showed that effective treatment to prevent germination of dinoflagellate cysts in seawater samples could be achieved with high concentrations of hydrogen peroxide (2% germination at 5,000 ppm and no germination at 10,000 ppm in a 24-h period). Motile cells of *Gymnodinium nagasakiense* have been killed in experiments using hydrogen peroxide absorbed by porous granules of calcium silicate and released in water. Cells were fatally damaged at concentrations of 3-6 mg L⁻¹ of hydrogen peroxide for 15-30 minutes (Miyazaki, Kurata et al. 1990; Ichikawa, Wakao et al. 1992; Montani, Meksumpun et al. 1995).

Toxicology

Algaestats have a low level of toxicity to fish, aquatic invertebrates and other non-target organisms under field use conditions due to its short residual time as an oxidizer and relatively low rates required to affect algae. Their application does not restrict use of water for swimming, fishing, irrigation, stock watering, drinking or domestic use following treatment (Applied Biochemists 2004).

Environmental fate and decomposition

Hydrogen peroxide has a half-life of less than 8 hours in an aquatic environment and can breakdown through hydrolysis, photolysis, anaerobic and aerobic metabolism, leaching and adsorption/desorption, and sediment dissipation (USEPA 2002).

Formulations and application methods

Trade marks for sodium carbonate peroxyhydrate products registered for applications to aquatic sites include GreenClean™, Pak 27™ and Phycomycin™. These are granular formulations that can be directly added to the water or dissolved into a liquid solution for spray or direct application to water.

An on-site hydrogen peroxide generator also exists and is produced by Eltron Water Systems LLC under the name of PeroxEgen™. This is a “turn-key”, mobile electrolytic technology that generates hydrogen peroxide (and optionally peracetic acid) in-situ for a variety of water treatment, advanced oxidation and cleansing applications by means of an electrochemical conversion of dissolved oxygen, which is carried out in a specially designed and patented electrochemical reactor. This system is being considered as an on-board treatment for ballast water (see more details below).

Use against unwanted aquatic plants, algae, bacteria and viruses

Ballast water tanks have been dosed with a concentration of peroxide sufficient to destroy cell integrity by attacking fatty acids within the membrane causing tissue damage.

Reported hydrogen peroxide concentrations required for the elimination of marine organisms range from 3 ppm for vegetative dinoflagellate cells of *Karenia mikimotoi* to over 140,000 ppm for *Bacillus subtilis* spores. A large discrepancy exists in the literature in relation to the concentration required to eliminate vegetative algal cells and dinoflagellate cysts (between 100 and 10,000 ppm), which may be due to different resistances of isolates utilized in these experiments, different organic loadings of seawater or different brands of hydrogen peroxide may vary in potency. Similarly, different exposure times used may explain this inconsistency. Elevating the pH of ballast water reduced the hydrogen peroxide concentrations required (1 ppm) to eliminate a range of invertebrate taxa including the ctenophore *Mnemiopsis leidyi*, the hydrozoa *Pennaria* sp., and a range of polychaetes, crustaceans, chordates and larval bivalves. Similarly, the efficacy of hydrogen peroxide can be enhanced by elevated temperature (up to 35°C) (Gregg, Rigby et al. 2009 and references therein)..

Bacterial spores are the most resistant marine organisms to hydrogen peroxide treatment requiring doses upward of 100,000 ppm for mortality to occur. The high concentrations needed for spore and dinoflagellate cyst inactivation would exclude hydrogen peroxide as a ballast water treatment as the cost involved are likely to be substantial. Additional concerns include hazards associated with the distribution, handling and onboard storage of large volumes of hydrogen peroxide. Hydrogen peroxide can however be produced *in-situ* by means of an electrochemical conversion of dissolved

oxygen, which is carried out in a specially designed and patented electrochemical reactor. PeroxEgen™ is an in-situ water treatment system claimed to be able to control ballast water organisms (including bacteria) by injecting low hydrogen peroxide concentrations (<100 ppm) into ballast water during intake (Eltron Water Systems LLC 2009). Efficacy data are not available. However, if this method can safely and effectively produce hydrogen peroxide concentrations able to inactivate resistant organisms, it may prove a feasible ballast water treatment (Gregg, Rigby et al. 2009 and references therein).

Selected Non-registered Aquatic Herbicides, Algaecides, Bactericides and Viruscides

Acetic Acid (Vinegar)

(See Chapter III for more information on acetic acid)

Uses against unwanted plants and algae

Sharp et al. (2006) tested the toxicity of 5% acetic acid, 30% brine, 5% citric acid and 5% sugar to the filamentous green algae *Cladophora* sp. and to mussel spat in *Mytilus edulis* (blue mussel) farms of Prince Edward Island, Canada. Mussel spat settling at the same time algal mats are blooming either do not attach to the collectors or become attached to algal filaments. These authors treated mussel collectors using a trough filled with the test solution and attached to a mussel work boat. Collectors were immersed in treatment agents for 15 and 30 seconds. The authors found that cellular damage in *Cladophora* plants exposed to acetic acid for 15 and 30 seconds and observed 15 minutes after treatment was comparable to that of *Cladophora* in the control collectors. Also, they observed that acetic acid had a negative effect on mussels (60% of mussel spat were unattached and/or gaping open 24 hours after the treatment) while the treatment with brine showed minimal impact on the mussels and caused significant damage to *Cladophora* cells.

In contrast, Forrest et al (2007) found that acetic acid was effective at killing *Cladophora* at 2% concentration after 3 minutes of immersion and at 4% after 1 minute of immersion.

Preliminary research trials by the USDA-ARS and the Invasive Spartina Project have shown that acetic acid may hold promise for killing rhizomes of smooth cordgrass (*Spartina alterniflora*) (Anderson 2003).

Acrolein

General information

Acrolein, also known as acrylaldehyde, allyl aldehyde, and 2-propenal is registered as a restricted use pesticide for the control of submerged and floating aquatic weeds and algae in irrigation canals as well as irrigation reservoirs in some states. In addition, acrolein is used as a biocide to kill bacteria that accumulate within the pipes of petroleum producing systems; for algae, weed and mollusk control in recirculating process water systems; for slime control in the paper industry; to protect liquid fuels against microorganisms; and to control sulfate reducing bacteria that produce corrosive hydrogen sulfide in oilfield water systems. It is also used for cross-linking protein collagen in leather tanning and for tissue fixation in histological samples (USEPA 2008; USEPA 2009).

Mode of action

The mechanism of toxic action of acrolein, as observed in mammalian and other systems, includes cell wall degradation and disruption of the cell's ability to inactivate toxic chemicals (Siemering, Hayworth et al. 2008). Other effects on cell energetics include reduction in intracellular ATP levels in tissue culture (Monteil, Prieur et al. 1999) and reduced beating activity of myocytes (Toraason, Luken et al. 1989).

Toxicology

Acrolein is acutely toxic to humans by inhalation, oral, and dermal exposures (Toxicity Category I for all routes). It is a potent irritant to the mucous membranes. Direct contact with liquid acrolein causes rapid and severe eye and skin irritation or burns. Dermal exposure to acrolein liquids or vapors may cause stinging of the eyes, lacrimation, and reddening, ulceration, or necrosis of the skin.

The evidence for the carcinogenicity of acrolein is equivocal, with a significant tumor incidence found in a single animal drinking water study. Glycidol, a metabolite of acrolein, is considered a probable human

carcinogen by the International Agency for Research on Cancer/ WHO (USEPA 2008).

In a recent report, the USEPA reviewed and expanded toxicological studies on acrolein and found that sufficient data were available to derive freshwater criteria for acrolein, but the lack of data precluded the estimation of saltwater criteria, a final plant value and a residue value (USEPA 2009).

Acute toxicity of acrolein was tested in fifteen species representing fourteen genera of freshwater organisms. Toxicity values (measured by species mean acute values, SMAV) ranged from 7 $\mu\text{g L}^{-1}$ for the African clawed frog (*Xenopus laevis*) to 5,920 $\mu\text{g L}^{-1}$ for an insect (*Peltoperia maria*). The white sucker (*Catostomus commersoni*) was the second most sensitive species tested, with mean acute value of 14 $\mu\text{g L}^{-1}$. Rainbow trout (*Oncorhynchus mykiss*) and the bluegill sunfish (*Lepomis macrochirus*) were the third and fourth most sensitive species tested, with SMAV of 16 and 27.19 $\mu\text{g L}^{-1}$, respectively (USEPA 2009 and references therein).

The least sensitive group of freshwater species to acrolein toxicity was invertebrates. The insect (*Peltoperia maria*) was the most tolerant to acrolein with a SMAV of 5,920 $\mu\text{g L}^{-1}$, followed by the midge (*Chironomus riparius*) with a SMAV of 510 $\mu\text{g L}^{-1}$, the snail (*Physa heterostropha*) with a SMAV of 368 $\mu\text{g L}^{-1}$, and the scud (*Gammarus minus*) with a SMAV of 180 $\mu\text{g L}^{-1}$. The snail (*Aplexa hypnorum*) and midge (*Tanytarsus dissimilis*) had SMAVs of >151 $\mu\text{g L}^{-1}$ acrolein each. The planktonic crustacean, *Daphnia magna*, was the most acutely sensitive invertebrate to acrolein with an SMAV of <39.76 $\mu\text{g L}^{-1}$ (USEPA 2009 and references therein).

No relationships have been demonstrated between water quality characteristics (such as hardness and pH) and toxicity. Acute toxicity has been tested with only four species of saltwater organisms. SMAV ranged from 100 $\mu\text{g L}^{-1}$ for the brown shrimp (*Penaeus aztecus*) to 500 $\mu\text{g L}^{-1}$ the mysid (*Americamysis bahia*) (USEPA 2009 and references therein).

Chronic toxicity of acrolein was tested in three freshwater species, but no saltwater species. The USEPA concluded that more studies are needed for marine animals in order to estimate acute and chronic saltwater criteria for acrolein. The most chronically sensitive freshwater species tested was the fathead minnow, *Pimephales promelas*, with a Chronic Value (CV) of 11.4 $\mu\text{g L}^{-1}$ based on reduced survival. Two additional studies with this species had measured CVs of 16.74 $\mu\text{g L}^{-1}$ and 22.14 $\mu\text{g L}^{-1}$, also based upon a survival endpoint. The remaining freshwater fish tested, the flagfish *Jordanella floridae*, had a CV of 25.92 $\mu\text{g L}^{-1}$ based on growth. The only freshwater invertebrate tested chronically was the cladoceran *Daphnia magna*, with a CV of 23.83 $\mu\text{g L}^{-1}$ based on survival. These data show that there is little difference in concentrations between the acute and chronic

effects of acrolein on *D. magna* and the tested fish species. It appears from available data that all tested freshwater species will be protected from adverse effects due to acrolein by the freshwater Chronic Value (USEPA 2009 and references therein).

Freshwater algae are affected by concentrations of acrolein as low as 36 µg L⁻¹, based on data for three species. The duckweed, *Lemna gibba*, was similarly affected at 72 µg L⁻¹ acrolein, as was the marine diatom, *Skeletonema costatum*, with a EC50 value of 28 µg L⁻¹ (USEPA 2009 and references therein).

Environmental fate and decomposition

Potential routes of acrolein degradation are via volatilization, microbial metabolism, and absorption into plants by cross-linking of protein. Degradation products include 3-hydroxypropanol, acrylic acid, allyl alcohol, propanol, propionic acid and oxalic acid. Acrolein forms several degradates. A unique feature of 3-hydroxypropanol is that it is in equilibrium with acrolein, and thus does not fully degrade via hydrolysis. Data are not available to characterize the rate of acrolein photolysis in water (USEPA 2009 and references therein).

The loss of acrolein by volatilization and degradation was measured in sealed bottles and tanks of freshwater. The amounts of acrolein dissipated after eight days were 34% from the tank and 16% from the bottles. The lack of turbulence in the tank reduced acrolein loss by volatilization to 1/20 of what would be expected if volatilization were controlled only by resistance in the gas phase and any discrete surface layers. The primary degradation reaction is reversible hydrolysis to β-hydroxypropionaldehyde, which is less volatile than acrolein (USEPA 2009 and references therein).

The fate of acrolein in freshwater was observed in buffered solutions and in natural channel waters. Equilibrium between acrolein and its degradation products was reached in the buffered solution following dissipation of 92% of parent compound, but in the natural channel waters there was no indication of equilibrium, with the dissipating reaction apparently continuing on to completion. Also, in the natural channel waters, the accumulation of a reaction (degradation) product was greater at higher initial acrolein concentration, and decay was rapid when acrolein concentrations fell below 2 to 3 mg L⁻¹. The initial period of slow decline preceding the rapid dissipation period was thought to be the result of microbiological processes. There was an 8- to 10-fold increase in the observed dissipation rate as compared to the expected rate in two of four flowing water channels, suggesting major losses in volatilization and absorption. A half-life of approximately seven h was observed for acrolein in freshwater, but the

dissipation rate was both concentration and temperature dependent. The presence of viable microbial populations also heavily influences the acrolein degradation rates in freshwater systems (USEPA 2009 and references therein).

In the marine environment, acrolein undergoes hydrolysis and oxidation to form β -hydroxypropanol and β -hydroxy propionic acid and half-life is estimated to be less than 20 h (USEPA 2009 and references therein).

Monitoring studies conducted after field application show that acrolein can be transported up to 61 miles from the point of application. Reported half-lives ranged from two to 20 h based on concentrations measured downstream of application. Field studies also determined that acrolein volatilizes from treated waters and represents a source of exposure to non-target animals through inhalation (USEPA 2009 and references therein).

Formulations and application methods

Magnicide H (aquatic herbicide, EPA Registration #:10707-9) and Magnicide B (biocide, EPA Registration #: 10707-10) are packaged as liquids and stored under an inert gas blanket. Each contains 95% acrolein as the active ingredient and have both been registered by Baker Petrolite Corporation. As an herbicide, acrolein is injected directly below the surface of moving water and moves with the flow of water killing weeds on contact in irrigation canals and holding ponds. Acrolein is also used as a biocide in water pumped into injection wells associated with petroleum production. Both the herbicide and biocide products are applied through a closed system. For herbicidal use in irrigation canals, the maximum single application concentration of acrolein is 15 ppm. The typical application rate is 8 ppm. For the biocide use, the maximum single application rate is 15 ppm. No maximum number of applications or minimum re-application intervals is specified on the labels (USEPA 2008).

Ammonium compounds and derivatives

(See Chapter II for more information on ammonium compounds)

Uses against unwanted plants and algae

Ammonium sulphate has been added to brackish water ponds to kill phytoplankton *Prymnesium parvum* and liquid ammonia was reported as a useful and inexpensive control agent in brackish water ponds to kill *P.*

parvum at temperatures lower than 20 °C and pH less than 8.5 (Kimor 2000).

Various quaternary ammonium compounds are registered by the USEPA for algae control in closed systems, such as industrial facilities.

Barley Straw

General information

The technique of using extracts from barley straw (*Hordeum vulgare*) for algae control was developed in the early 1990's in England, where it is widely applied for these purposes in many bodies of water, including large reservoirs and canals (Lembi 2002; Ferrier, Butler et al. 2005). In the US, barley straw has been applied experimentally for algae control in ponds and other small enclosed water bodies.

Mode of action

In general, it is thought that fungi decompose the barley in water, which causes a chemical to be released that prevents the growth of the algae. The specific chemical that works as the active ingredient has not been identified, although studies suggest oxidized polyphenolics and hydrogen peroxide are two decomposition products. It is not clear whether the chemical is exuded from the barley itself or if it is a metabolic product produced by the fungi. The activity of barley straw is usually described as being algistatic rather than algaecidal (Lembi 2002).

Selectivity

A short-term laboratory study was conducted to investigate the effect of barley straw in controlling several common phytoplankton and cyanobacterial species. Following a one-month incubation of barley straw in coarsely filtered fresh river and brackish river waters, the growth of six autotrophic taxa was followed in culture. Barley straw slurry reduced the yield of three taxa (*Ankistrodesmus falcatus*, *Chlorella capsulata*, *Isochrysis* sp.) in comparison with cultures not receiving the slurry. Although no significant changes in growth were detected with three other taxa (*Cyclotella* sp., *Ulothrix* sp., freshwater *Pseudanabaena* sp.), some patterns indicated potential impacts of the barley straw. First, a higher addition of straw to *Cyclotella* sp. resulted in a lower biomass accumulation than in cultures receiving lower levels. Second, the bloom-forming dinoflagellate

Prorocentrum minimum was apparently stimulated at low barley straw levels, perhaps suggesting conditions associated with the straw (metals-chelation, bacterial-produced nutrients) might stimulate dinoflagellate growth. Third, species shifts were observed in two of the cultures, with barley straw favoring shifts from *Isochrysis* to a *Cyclotella* sp. – *Thalassiosira* sp. mixture and shifts from *Pseudanabaena* to a *Pseudanabaena* – *Scenedesmus* mixture. These results provide new records for the susceptibility of freshwater and brackish phytoplankton taxa to barley straw exposure, including species-specific responses and shifts in species dominance in mixed assemblages (Brownlee, Sellner et al. 2003).

In a study to determine the efficacy of barley straw liquor in controlling algal growth of 12 freshwater species of nuisance algae, researchers found that the product inhibited the growth of three common algae: *Synura petersenii*, *Dinobyron* sp., and *Microcystis aeruginosa*. In contrast, a significant increase in growth was observed in populations of *Selenastrum capricornutum*, *Spirogyra* sp., *Oscillatoria lutea* var. *contorta*, and *Navicula* sp. in the presence of barley straw liquor. Growth of the remainder species, *Ulothrix fimbriata*, *Scenedesmus quadricauda*, *Chlorella vulgaris*, *Anabaena flos-aquae*, and *Synedra* sp. showed no significant difference from controls. In addition, parallel field studies were conducted. Four of six ponds were treated with barley straw. Monitoring of chlorophyll *a* levels in these ponds for one growing season, showed that while phytoplankton populations in all ponds decreased in midsummer, the phytoplankton biomass in treated ponds did not differ significantly from that of control ponds, suggesting that the application of barley straw had no effect on algal growth in these systems (Ferrier, Butler et al. 2005).

Barley straw extract was also tested as a suppressant for *Prymnesium parvum*, a harmful algae whose blooms can cause fish kills in brackish waters. Barley straw extract did not reduce the exponential growth rate, endpoint density, or toxicity to fish of *P. parvum* (Grover, Baker et al. 2007).

Uses against unwanted plants and algae

Mostly experimental. See selectivity section.

Chlorination

(See Chapter II and III for more information on chlorine compounds and derivatives)

Uses against unwanted plants, algae and bacteria: Chlorination has been shown to eliminate aquatic organisms but the concentration required varies

considerably with different organisms. Vegetative algal cells and free-living zooplankton can be killed at concentrations of 1-100 ppm; however resistant organisms, such as dinoflagellate cysts, zooplankton resting stages and *Bacillus subtilis* spores require considerably higher concentrations (486-2,500 ppm) (Gregg, Rigby et al. 2009 and references therein).

Free chlorine levels between 0.2-1.0 ppm are required to control phytoplankton growth. Williams et al. (1982) as cited in Bolch & Hallegraeff (1993) required free chlorine concentrations (as sodium or calcium hypochlorite) or 20ppm over 24 hours to kill small shrimp and larval fish in ballast water samples (McEnnulty, Bax et al. 2001).

Laboratory studies have found that electrolysis of natural seawater produces NaOCl at concentrations of 0.5ppm rapidly reduced populations of five red tide dinoflagellates including *Gymnodinium sanguineum*. Ciliates and copepods were not affected (Carpenter et al. 1972; Boyd 1996; Kim et al. 2000).

Electrolysis of natural seawater produces NaOCl at concentrations and has been used to control dinoflagellates in laboratory studies and in ballast water (Carpenter et al. 1972; Boyd 1996; Kim et al. 2000), *Caulerpa taxifolia* (Boudouresque et al. 1996) and fouling in ship water piping systems (Ganti and Kalyanasundaram 1975). Chlorination is also used to control fouling bivalves and prevent settlement in freshwater reservoirs, treatment works, power stations and water ways and raw water supply systems, estuarine waterworks and seawater cooled power stations (Morton et al. 1976; Rajagopal et al. 1997b).

Hypochlorite produced *in situ* using an electrolytic cell and applied at concentrations of ~1,000 ppm for 10 minutes is reported to have a lethal effect on *Caulerpa taxifolia* after 96 h. However, recovery occurred after 8 days, i.e. the effect of hypochlorite was merely temporary (Boudouresque et al. 1996).

Chlorine (sodium hypochlorite, sodium azide) and electrolysis of seawater to produce chlorine (NaOCl) has been used on ballast water to disable toxic algal cysts and vegetative cells including *Gymnodinium catenatum* (Carpenter et al. 1976; Bolch and Hallegraeff 1993; Montani et al. 1995; Hallegraeff et al. 1997; Kim et al. 2000).

Sodium hypochlorite has been trialed as control agent for the brown seaweed *Sargassum muticum* in England and France but the low specificity and persistence in the environment made it unsuitable for this use (Critchley et al. 1986; Belsher 1991; Ribera and Boudouresque 1995).

Dinoflagellate cysts require a much greater concentration of chlorine than vegetative cells (Bolch and Hallegraeff 1993). Estimates by Rigby et al. (1993) based on chlorine concentrations required to kill *Gymnodinium* cysts found 400 tonnes of an industrial solution of 12.5% sodium hypochlorite was needed to treat a 50,000 tonne ballast water tank. Apart from being prohibitively expensive (except when produced from seawater by a ship-board generator) and causing environmental problems as the port of discharge, the high sediment load of ships' ballast tanks would considerably reduce the available free chlorine levels (Bolch and Hallegraeff 1993).

Electrolysis of seawater to produce NaOCl could be used in closed systems and power station cooling waters to control phytoplankton blooms and fouling species, but would be impractical on a large scale in open ocean situations because of the amounts of chlorine required. Half the NaOCl produced by electrolysis degraded to harmless NaCl within 2 h in bright sunlight (Kim et al. 2000).

Application methods vary including gas injection, solutions pumped underneath black plastic used to smother the sediments or through water conduits or placing bags of solution around target species.

An application of 5–10 mgL⁻¹ for 1–24 h is sufficient to kill harmful bacteria and all other organisms (Piper et al. 1998); the exact amount depends on the target species and the local water chemistry, particularly the amount of organic matter present.

Bacterial susceptibility to chlorine varies greatly, with free-living gram-positive and gram-negative bacteria highly susceptible, whereas acid-fast bacteria, bacteria associated with crustaceans and bacterial spores require higher doses. For example, *Bacillus subtilis* spores require a chlorine concentration of 500 ppm for inactivation to occur, whereas *Vibrio* sp. has been completely eliminated at 5 ppm. Similarly, doses of 10 ppm were effective in killing free-living *Vibrio cholerae*; however this concentration was insufficient to destroy *Vibrio*'s adhering to crustaceans, probably due to an increase in organic matter, which reduces the bactericidal properties of chlorine. Another study found toxigenic strains of *V. cholerae* required a much higher dose of 100 ppm for the control of free-living cells, with even higher doses (800 ppm) required to achieve satisfactory control of attached *V. cholerae*, yet even at this concentration, regrowth was apparent within 20 min. Chlorination at 800 ppm was not effective against *V. cholerae* when applied to 100% or 10% strength ballast water suggesting that attachment to organisms and particulate matter enhanced their survival (Gregg, Rigby et al. 2009 and references therein).

Electrolyzed seawater may be used to generate chlorine for shipboard ballast water treatment and may act to reduce the cost of chlorine

treatment. Efficacy values vary: in one study, a 3 ppm chlorine concentration generated electrolytically from seawater was found to reduce bacteria by more than 99.999% and reduce phytoplankton and mesozooplankton by 99%. Conversely, in another study, only 72% of total phytoplankton was killed when raw seawater was treated by electrolysis with an initial chlorine concentration of 4 ppm (Gregg, Rigby et al. 2009 and references therein).

The use of chlorine dioxide (ClO_2) in ballast water disinfection is considered advantageous over chlorine for several reasons (Gregg, Rigby et al. 2009, see Chapter II for more details). The majority of chlorine dioxide work has been conducted on freshwater organisms, with only few studies available for marine systems. Nonetheless, results indicate that it is an excellent bactericide and sporicide, and is more effective in controlling vegetative bacteria and viruses compared with chlorine. For example, a range of viruses could be controlled at a concentration of 1-7 ppm chlorine dioxide, whereas a 7 ppm dose of chlorine had no observable effects. Efficacy studies conducted in seawater have found that vegetative cells of the dinoflagellates *Alexandrium catenella*, *Gymnodinium catenatum*, *Protoceratium reticulatum* and *Scrippsiella trochoidea* are eliminated at 25 ppm after 2 h; and sexual resting cysts of *G. catenatum* and *P. reticulatum* are inactivated following 2 wk exposure to 50 ppm. A 3 ppm concentration chlorine dioxide produced by a generator against *Artemia* cysts can reduce the hatching rate by 97% (Gregg, Rigby et al. 2009 and references therein).

Newer chlorine dioxide generators are suggested to produce no chlorinated by-products or toxic residual material at discharge. The Ecochlor® Ballast Water Treatment System generates chlorine dioxide using the Eka Chemical Purate® technology, a method that differs from conventional chlorine dioxide generation methods in that it does not involve or create free available chlorine or chlorinated by-products (<http://www.ecochlor.com>). The treatment system uses Purate® solution (containing 40% sodium chlorate and 8% hydrogen peroxide) and sulphuric acid to produce ClO_2 , which is injected into ballast water during intake (Gregg, Rigby et al. 2009 and references therein).

Laboratory experiments demonstrated that an initial ClO_2 concentration of 5 ppm was effective at eliminating bacterial and planktonic populations to the extent that no regrowth was observed. Shipboard studies on the biological efficacy of the treatment system as well as degradability and corrosion tests were carried out onboard the MV Atlantic Compass in 2005. Results from these experiments showed that the application of 5 ppm of ClO_2 immediately eradicated 100% of zooplankton, reduced the abundance of total coliform bacteria, *Vibrio* colonies and *E. coli* to non-detectable levels within the first 24 h, and virtually eliminated all phytoplankton biomass.

Some recovery of bacteria and phytoplankton was observed after 5 days indicating that the treatment did not eliminate 100% of organisms. This regrowth was attributed to the presence of a biofilm in the ballast tanks which provided refuge for the organisms that survived (Gregg, Rigby et al. 2009 and references therein).

Degradability studies conducted during the shipboard trials determined that ClO₂ remained active in the ballast tanks for several hours but degraded at a rate that resulted in no residual ClO₂ in the ballast water at the time of discharge. For example, an initial dosage of 5 ppm left no residual ClO₂ after 20-24 h when the temperature of ballast water was between 10 and 12 °C, whilst an initial dosage of 8 ppm in 25-26 °C ballast water left no trace after 24-25 h. Furthermore, corrosion tests conducted in ballast tanks indicated that ClO₂ had no adverse effect on corrosion, in fact a slight decrease in corrosion was observed in the treated tanks during one experiment. It should be noted that the corrosion tests were only carried out for a period of 28-32 days. Much longer investigations are required to fully understand the effect of the treatment system on corrosion rates.

Diuron

General information

Diuron is a substituted urea herbicide registered for pre- and post-emergent treatment of both crop and non-crop areas, as a mildewcide and preservative in paints and stains, and as an algacide in commercial fish production, residential ponds and aquariums.

Mode of action

The mechanism of herbicidal action is the inhibition of photosynthesis (USEPA 2003).

Toxicology

Diuron is considered of low acute toxicity to birds, mammals, freshwater fish, estuarine fish, freshwater invertebrates, and estuarine invertebrates, and low acute toxicity to humans by the oral, dermal, or inhalation exposure routes. Diuron is not an eye or skin irritant, and not a skin sensitizer. A rat metabolism study indicated that diuron is rapidly absorbed and metabolized within 24 hours post-dose at the low dose and within 48 hours post-dose at the high dose (USEPA 2003).

Environmental fate and decomposition

Diuron is persistent and stable to hydrolysis. Calculated half-lives in aqueous and soil photolysis are 43 and 173 days, respectively. Half lives in laboratory aerobic and anaerobic soil metabolism studies are 372 and 1000 days, respectively. However, in a viable laboratory aquatic system, decomposition occurred with half-lives of 33 and 5 days in aerobic and anaerobic systems, respectively. In soil, the half lives of diuron and its degradate DCPMU range from 73 to 139 days and 217 to 1733 days, respectively.

Uses against unwanted plants and algae

In situ tests, tried various methods of applying herbicides including diuron to *Undaria pinnatifida*, such as injection into the stipe or midrib, applying a gel formulation, attaching a sponge saturated with active substance to the thallus, and applying compounds inside a bag enclosing the thalli. These methods proved to be labor-intensive and had no appreciable impact (Sanderson 1996). Laboratory studies on *Ecklonia radiata* using diuron found very high concentrations were needed to inhibit zoospore germination (Burridge and Gorski 1998).

The USEPA has authorized Mississippi catfish growers to use diuron to combat blue-green algae that, when present in catfish ponds, is linked to off-flavor fish. Diuron was approved by the USEPA under a FIFRA Section 18 emergency exemption label.

Glutaraldehyde

General information

Glutaraldehyde is an organic biocide which has been proposed, either on its own or in combination with surfactant, for the treatment of vessels carrying little or no ballast to control organisms present in ballast tank residues and sediment (Sano et al. 2003, 2004).

Glutaraldehyde is most active at higher temperatures and above a pH of 7.5 (Sagripanti and Bonifacino 1996). As ballast tank pH generally varies from 4.2 to 8.6, this may limit the application of glutaraldehyde, unless the treatment system can account for this strong pH response. Glutaraldehyde is corrosive in its concentrated form, but is not considered to pose any corrosion problems in the diluted form proposed for ballast water treatment.

Environmental fate and decomposition

The biodegradation of glutaraldehyde relies on its digestion by microbes. As glutaraldehyde is a biocide, the concentration required to remove ballast water organisms is also likely to inhibit bacterial growth and metabolism, however once discharged the concentration will decline as the ballast water is dispersed into the aquatic environment. The biodegradability of glutaraldehyde in seawater has been tested and is a complex issue varying with factors such as initial concentration, nutrient status and microbe concentration. Leung (2001) indicates that glutaraldehyde is considered readily biodegradable in the freshwater environment and has the potential for biodegradation in the marine environment.

Use against unwanted aquatic plants and algae

It has been demonstrated to successfully control the marine bacterium *Vibrio fischeri* at a concentration of 8- 14 ppm; however doses of 20,000 ppm are required to inactivate *B. subtilis* spores (Sano et al. 2003; Sagripanti and Bonifacino 1996).

Observations by Sano et al. (2003) indicate that some ballast water organisms may be resistant to glutaraldehyde treatment; consequently eliminating most organisms may require a concentration of 500 ppm. Pre-treatment of fine filtration may act to reduce this concentration as the amount of sediment and organic carbon present in ballast tanks is likely to impact on the efficacy of glutaraldehyde. In situations where higher amounts of sediment exist in relation to water, the ability of glutaraldehyde to penetrate these sediments and kill any viable organisms is likely to be limited (Sano et al. 2003). In this situation, even higher doses may be required for adequate organism removal. The high glutaraldehyde concentration required to control ballast water organisms induces three important limitations with respect to ballast water treatment. Firstly, the cost of treatment will be prohibitively expensive; secondly, the potential for detrimental environmental impacts is increased; and finally, occupational health and safety risks increase.

Sano et al. (2003) suggest the cost of glutaraldehyde treatment would equate to US\$25 per tonne of ballast water, thus limiting the treatment to vessels with small quantities of ballast water and sediment. The increased risk of environmental impacts will relate to the time required for glutaraldehyde to degrade and the amount of dilution that takes place prior to release into the receiving port (Leung 2001; Sano et al. 2003).

Menadione (SeaKleen®)

General information

Menadione is a water-soluble form of Vitamin K3, which belongs to the chemical class of naphthoquinones, and has been shown to be toxic to a wide range of freshwater and marine organisms.

Several other naphthoquinone compounds are currently being investigated as potential ballast water biocides. These include juglone, plumbagin and menadione nicotinamide bisulphate (Faimali et al. 2006; Wright et al. 2007b). Like menadione, juglone and plumbagin are natural plant products, with juglone isolated from the black walnut tree *Juglans nigra*, and plumbagin, a compound found in members of the sea lavender family, Plumbaginaceae (Wright et al. 2007b). Both products have been shown to exhibit greater bactericidal activity and overall toxicity to aquatic organisms compared to menadione (SeaKleen®), however menadione was considered to be favorable biocide for ballast water treatment as the production cost of menadione is less than 2% the cost of either juglone or plumbagin (Wright et al. 2007a). Nonetheless, further work should assess the degradability of juglone and plumbagin and their ability to inactivate resistant marine organisms, such as dinoflagellate resting cysts.

Menadione nicotinamide bisulphite is a synthetic derivative of menadione (Faimali et al. 2006). Preliminary screening of its efficacy against marine organisms found that it can effectively eliminate a variety of ballast water organisms in the absence of light. Zooplankton larvae, including *Artemia salina* nauplii, *Balanus amphitrite* nauplii, *Mytilus galloprovincialis* larvae and *Tigriopus fulvus* larvae were completely eliminated at concentrations of 0.5 to 5 ppm, growth of the green alga *Chlorella minutissima* was inhibited at 0.5 ppm and germination of dinoflagellate cysts (*Scrippsiella trochoidea*) was reduced to 30% (compared to 90% in controls) after exposure to 5 ppm (Faimali et al. 2006). The product displayed a variable effectiveness against bacteria (1 to >64 ppm required to inhibit regrowth) and was not as effective against *Alexandrium catenella* (EC 50=32 ppm) (Faimali et al. 2006). The major advantage this compound has over its parent molecule (menadione) is that it is highly biodegradable. Faimali et al. (2006) imply that menadione nicotinamide bisulphite has a half life of 48 h under dark conditions and <6 h under light conditions compared to 1,500 h for menadione when prepared in drinking water.

Selectivity

Laboratory testing has proved SeaKleen® to be toxic to aquatic algal species (*Chlorella* sp., *Isochrysis galbana*, *Neochloris* sp., *Tetraselmis suecica*), vegetative dinoflagellates (*Alexandrium catenella*, *A. tamarense*, *Gymnodinium catenatum*, *Karenia brevis*, *K. brevisulcata*, *Karlodinium veneficum*, *Prorocentrum minimum*, *Protoceratium reticulatum*, *Scrippsiella trochoidea*), dinoflagellate temporary cysts (*Glenodinium foliaceum*), raphidophytes (*Chattonella marina*) and zooplankton (*Crassostrea virginica* larvae, *Cyprinodon variegates*, *Dreissena polymorpha* larvae, *Leptocheirus plumulosus*, *Mytilus galloprovincialis*) at concentrations ranging from 0.5 to 2 ppm (Wright and Dawson 2001; Cutler et al. 2004; Gregg and Hallegraeff 2007).

Toxicology

Apart from its broad toxicity, the manufacturer suggests that SeaKleen® is an attractive ballast water biocide because it is apparently of low toxicity to mammals, birds and species of fish,

Environmental fate and decomposition: it has a short half-life causing it to degrade to harmless products within days, it has no known corrosive properties, and it is relatively cost-effective (Wright and Dawson 2001). Inconsistencies also exist in the literature concerning the degradability of SeaKleen®. Herwig and Cordell (2004) and Wright et al. (2007a) reported a half life of 18-30 h for SeaKleen®, yet Cutler et al. (2004) found that SeaKleen® degraded to only 21% of the initial concentration in darkness in seawater without any organisms after 28 d. The latter authors suggest that degradability is faster under light conditions and in the presence of biological material; however, Gregg and Hallegraeff (2007) found that the degradation of 4 ppm SeaKleen® was minimal after 15 wk and was not influenced by the presence of ballast tank sediment, biological matter or light conditions. Faimali et al. (2006) also report that exposure to light failed to accelerate the degradation rate of SeaKleen®. These authors indicate that a 10 ppm concentration of menadione in drinking water has a half life of 1,500 h under both light and dark conditions and takes >5,000 h to totally degrade.

Formulations and application methods

SeaKleen® is a patented biocide developed by Garnett, Inc. Atlanta and manufactured by Vitamar Inc. Memphis.

Use against unwanted aquatic plants and algae

Full-scale shipboard trials of SeaKleen® were conducted onboard the USS Cape May in Baltimore Harbour in 2001. Results from the tests indicate that dosing ballast tanks with a concentration of 2 ppm SeaKleen® resulted in overall zooplankton mortalities of 99 and 100% after 24 and 48 h (Wright et al. 2004).

Phytoplankton were controlled with SeaKleen® concentrations as low as 1 ppm after 24 h, however the effectiveness against bacteria was not clear (Wright et al. 2004, 2007a). Some disagreement exists regarding the bactericidal properties of the product. Wright and Dawson (2001) and Cutler et al. (2004) suggest that SeaKleen® is extremely effective against bacteria and can eliminate *Escherichia coli* and *Vibrio fisheri* at a concentration of 1 ppm. Conversely, mesocosm experiments conducted at the University of Washington claimed that SeaKleen® at 2 ppm had no observable effect against culturable bacteria (Herwig and Cordell 2004) and Gregg and Hallegraeff (2007) required concentrations of 50-200 ppm to inhibit regrowth of *E. coli*, *Listeria innocua*, *Staphylococcus aureus* and *Vibrio alginolyticus*.

It has been suggested that the use of SeaKleen® may be advantageous in situations where water turbidity is high or to treat residual ballast tank sediments due to a low binding affinity to particulate matter (Wright et al. 2007a). Several studies indicate that SeaKleen® does retain its activity in the presence of sediment but it is less effective against resistant resting stages and sediment dwelling organisms. For example, Gregg and Hallegraeff (2007) found that the biocidal effect of SeaKleen® was not influenced by the presence of sediment but the product failed to kill resistant resting cysts of the toxic dinoflagellate *Alexandrium catenella* at 5 times the recommended dose (10 ppm) in sediment-free trials. Additionally, Sano et al. (2004) controlled the amphipod *Hyaella azteca* at comparable SeaKleen® concentrations in both sediment-free samples (2.5 ppm) and samples containing a 1:4 sediment to water ratio (3.5 ppm), but 88 ppm was required to control the burrowing oligochaete *Lumbriculus variegates* in the 1:4 sediment to water ratio compared to 1.8 ppm required in the water-only exposures. These findings indicate that SeaKleen® may provide an effective treatment against organisms in the water column when ballast water contains a high suspended sediment load. While effective control of resistant resting stages and sediment-dwelling organisms might be possible, the required concentrations would be likely to make the treatment prohibitively expensive and may pose environmental problems due to the discharge of toxic ballast water and residual sediment.

The estimated cost of SeaKleen® is approximately US\$0.20 per tonne when applied at a concentration of 2 ppm, which may limit the use of this biocide to vessels with small or moderate ballast capacities. Additional concerns that may limit the use SeaKleen® as a routine ballast water

treatment option include the possible discharge of toxic ballast water due to low degradability of the biocide and the limited effectiveness against bacteria (Gregg and Hallegraeff 2007).

Peracetic acid (Peraclean®)

General information

Peracetic acid is an organic biocide suggested as a potential ballast water treatment due to its broad-spectrum activity and lack of undesirable by-products. Very little data exists on the efficacy of peracetic acid in marine systems; however it has been documented to control coliform bacteria in sewage sludge at a concentration of 6-8 ppm, and bacterial spores at 300 ppm (Baldry and French 1989; Sagripanti and Bonifacino 1996). Activity is not affected in the presence of suspended solids and organic matter, however is affected strongly by pH.

Environmental fate and decomposition

The manufacturer indicates that Peraclean® Ocean has a half-life of only 4 h in unfiltered seawater and recommends a retention time of 1-2 d in ballast tanks due to its rapid degradation. One of the added advantages over peracetic acid disinfection is that it is most active at pH values of 5-7, but displays good activity up to a pH of 9 (Fuchs et al. 2001).

Use against unwanted organisms

Sagripanti and Bonifacino (1996) found the biocide to be most active at a pH of less than 3, while activity is lost above a pH of 8.

This finding may limit the application of peracetic acid as a ballast water biocide, however Degussa AG of Germany has developed a ballast water treatment product composed of peracetic acid and hydrogen peroxide, with the trade name Peraclean® Ocean. It is suggested to be effective against a broad range of microorganisms including bacteria, spores, phytoplankton, aquatic invertebrates and fish eggs at concentrations of 50-400 ppm and exposure times of 2- 72 h (Fuchs et al. 2001; Fuchs and de Wilde 2004). Apart from broad toxicity, Peraclean® Ocean is claimed to be effective over a wide range of conditions, to be relatively unaffected by organic matter and readily biodegradable and decompose into acetic acid, oxygen and water.

Peraclean® Ocean has been developed as either a standalone treatment or as the final stage of a treatment system that uses a combination of

technologies. Lab-scale testing has shown that the product is capable of eliminating bacteria, vegetative marine microalgae, dinoflagellate cysts and several different development stages of the brine shrimp *Artemia salina*. Gregg and Hallegraeff (2007) eliminated vegetative cells of the marine dinoflagellates *Alexandrium catenella*, *Gymnodinium catenatum*, *Protoceratium reticulatum* and *Scrippsiella trochoidea* at 50 ppm and killed the green flagellate *Tetraselmis suecica* at 100 ppm after 48 h exposure. Fuchs et al. (2001) killed the green alga *Chlorella* within 48 h at 200 ppm, however higher concentrations (up to 1600 ppm) did not result in more rapid mortality (Fuchs et al. 2001). This would suggest that exposure time is a very important aspect of the biocidal action of Peraclean® Ocean for the control of algae. In addition, Peraclean® Ocean inhibited bacterial regrowth of *Escherichia coli*, *Staphylococcus aureus*, *Listeria innocua* and *Vibrio alginolyticus* at 125-250 ppm and could completely inactivate *Artemia salina* cysts and resting cysts of the marine dinoflagellates *Gymnodinium catenatum*, *Alexandrium catenella* and *Protoceratium reticulatum* at 350-700 ppm (Fuchs and de Wilde 2004; Gregg and Hallegraeff 2007). Full-scale shipboard and land-based testing has proved that Peraclean® Ocean is an effective biocide for the control of a wide range of planktonic organisms and marine bacteria in both freshwater and seawater (Wright et al. 2004; Veldhuis et al. 2006; de Lafontaine et al. 2008a).

Veldhuis et al. (2006) examined the effectiveness of a full-scale land-based ballast water treatment system that used Peraclean® Ocean as a final disinfection step. Peraclean® Ocean was applied to estuarine water at a concentration of 150 ppm and resulted in the elimination of all zooplankton and phytoplankton but bacterial regrowth was observed after 6 to 10 days indicating that the biocide did not result in full sterilization (Veldhuis et al. 2006). De Lafontaine et al. (2008a) treated freshwater ballast onboard the MV *Canadian Prospector* using a Peraclean® Ocean concentration of ~100-150 ppm and observed a >90% reduction in free floating microorganisms and phytoplankton after 5 d. The treatment also showed a lethal effect on fish with 100% mortality achieved in less than 19 h for a range of cold water fish species, however the treatment was found to be ineffective against adult zebra mussels and some organisms buried in ballast sediments were not affected by a 5 d exposure to ~100-150 ppm of Peraclean® Ocean (de Lafontaine et al. 2008a).

Although the manufacturer indicates that Peraclean® Ocean-treated ballast water may be safely discharged after 1-2 d, degradability studies have shown that this biocide may degrade at a slow rate resulting in the discharge of potentially toxic ballast water into the marine environment. Results from the shipboard trials conducted by de Lafontaine et al. (2008a) suggest a retention time of 15-29 d is required prior to discharge when

using Peraclean® Ocean at a concentration of 100-150 ppm for treating freshwater ballast due to the presence of toxic residues, and studies conducted in marine harbor water recommend a retention time of >6 d (Veldhuis et al. 2006). Biodegradability studies by Gregg and Hallegraeff (2007) found that 200 ppm Peraclean® Ocean concentrations degraded to a level non-toxic to marine microalgae in 2-6 wk. Degradation occurred more rapidly when exposed to light and ballast tanks sediments, whereas filtered seawater, humus-rich seawater, relatively clear freshwater and a lack of light appear to be the worst conditions for the degradation of Peraclean® Ocean (Gregg and Hallegraeff 2007; de Lafontaine et al. 2008b). The cost for this type of treatment is suggested to be in the vicinity of US\$0.20-0.30 per tonne of ballast water (Rigby and Taylor 2001). Apart from being expensive for use onboard ships with large ballast capacities, some concerns still exist on the routine use of Peraclean® Ocean with regard to potential residual toxicity of treated ballast water (e.g. when the holding time for degradation onboard is shorter than recommended), reduced effectiveness in the presence of sediments, the need to store chemicals onboard and possible ship corrosion.

Metsulfuron-methyl

General information

Metsulfuron-methyl is compound used as a selective pre- and post emergence herbicide for broadleaf weeds and some annual grasses. The most common uses of metsulfuron-methyl include wheat, barley, rye, and pastures.

Mode of action

Metsulfuron-methyl is a systemic sulfonylurea compound with foliar and soil activity and works rapidly after it is taken up by the plant. Sulfonylurea herbicides act specifically by inhibiting the enzyme acetolactate synthase, which is involved in the biosynthesis of valine, leucine and isoleucine in both plants and microorganisms. Soon after herbicide application, plant cell division stops quickly and plant death occurs within one to three weeks (Chiconela, Koschnick et al. 2004).

Selectivity

A study that tested metsulfuron-methyl's phytotoxicity on six wetland species found that torpedograss (*Panicum repens*), knotgrass (*Paspalum distichum*), and paragrass (*Brachiaria mutica*) were not affected by metsulfuron-methyl when applied at rates of up to 140 g ha⁻¹; soft-stem bulrush (*Scirpus validus*) growth was reduced at the highest application rate. In contrast, metsulfuron methyl had a dramatic effect on pickerelweed and arrowhead (*Sagittaria lancifolia*), producing essentially complete control of arrowhead and over a 90% reduction in pickerelweed biomass at rates of 17.5 g ha⁻¹. It required an application of 140 g ha⁻¹ to provide total control of pickerelweed in this study, but the authors believe incomplete coverage of pickerelweed may have occurred at the lower rates. Metsulfuron-methyl was very selective, controlling the broadleaf monocot species but not affecting the perennial grasses. The authors thus concluded that this herbicide would be a good candidate for use in selectively controlling certain broadleaf species in lake drawdown and restoration sites if appropriate labeling could be obtained (Chiconela, Koschnick et al. 2004).

In addition, the sensitivity of 12 aquatic plant species (i.e. *Elodea canadensis*, *Callitriche platycarpa*, *Potamogeton crispus*, *Ceratophyllum demersum*, *C. submersum*, *Batrachiumt richophyllum*, *Berula erecta*, *Sparganium emersum*, and *L. trisulca*—and 1 floating aquatic macrophyte — *Spirodela polyrrhiza*) to metsulfuron-methyl was tested in microcosm experiments under two growth conditions. Fast-growing species with a small exposed leaf area proved to be more sensitive to the herbicide than slow growing species with a large exposed leaf area, which was believed to be primarily due to variations in growth rates rather than to variations in exposed leaf area. The aquatic plants displayed high tolerance in growth to metsulfuron compared with the sensitive crop oil-seed rape. Hence, possible spray-drift events and leaching of the herbicide applied at agricultural rates are not considered to have a large impact on the growth of the aquatic flora tested (Cedergreen, Streibig et al. 2004).

In a study of short-term (2 weeks) effects of metsulfuron-methyl alone and in combination with the insecticide cypermethrin in freshwater enclosures (80 L), researchers found that the root growth of the macrophyte species *Elodea canadensis* and *Myriophyllum spicatum* decreased following exposure to the lowest concentration of metsulfuron methyl tested. Metsulfuron-methyl exposure resulted in a decreased pH in the aquatic enclosure at the lowest concentration tested, which is most likely a further indication of decreased macrophyte primary production. The biomass of periphytic algae growing on the leaves of *M. spicatum* increased in the enclosures exposed to metsulfuron methyl. The exposure to metsulfuron-methyl did not alter the biomass or the species composition of the phytoplankton community (Wendt-Rasch, Pirzadeh et al. 2003).

Toxicology

Metsulfuron-methyl has very low toxicity in mammals; low dermal toxicity in tests with rabbits; low inhalation toxicity in rats; moderate but reversible eye irritation has been seen in rabbits, and mild skin irritation has been observed in guinea pigs. No skin sensitization has been observed in guinea pigs (EXTOXNET 1995).

Formulations and application methods:

Metsulfuron-methyl can be used with other foliar herbicides and is commercially available in the form of dry flowable formulations (EXTOXNET 1995).

Uses against unwanted plants and algae

In 2003, the South Florida Water Management District (SFWMD) was granted with a FIFRA Section 24(c) special local need registration to use Escort XP (a.i. metsulfuron methyl) for controlling old world climbing fern (*Lygodium microphyllum*) in aquatic environments in Florida. Old world climbing fern is an aggressive perennial vine that has invaded cypress stands, pine flatwoods, wet prairies, sawgrass marshes, mangrove communities, and Everglades tree islands in Florida. The most effective product for controlling the vine registered for direct application to water was the glyphosate-based herbicide RodeoT, a broad spectrum herbicide that injures or kills many nontarget species. SFWMD performed several experiments and the process of obtaining the Section 24(c) registration took more than a decade.

Lime compounds and derivatives

(See Chapters II and III for more information on lime)

Uses against unwanted plants and algae

In Canada, 4 % hydrated lime solutions were used in an attempt to kill *Codium fragile* and prevent this alga's translocation between areas. The alga was very difficult to eradicate using these treatments although it took a long time to assess their efficacy (MacNair and Smith 1999).

In situ tests, tried several methods of applying lime to *Undaria pinnatifida*, including the injection of lime into the alga's stipe or midrib, applying a gel

formulation, attaching a sponge saturated with active substance to the thallus, and applying compounds inside a bag enclosing the thalli. However these methods proved to be labor-intensive and had no appreciable impact (Sanderson 1996).

The macroalgae *Macrocystis pyrifera*, *Gigartina canaliculata*, *G. leptorhynchos*, and *Egregia laevigata*) survived heavy applications (5- 50 tonnes ha⁻¹) of quicklime (Locke, Doe et al. 2009).

Phosphorus inactivation products

General information

A direct relationship usually exists between the amount of phosphorus in a contained water body and the amount of algae growing in this water. As phosphorus levels increase, the amount of algae increases too. At very high levels of phosphorus, other nutrients or light may limit the growth of algae. Long-term management of excessive algae may involve the removal of phosphorus sources to the water body as it eliminates a key algal nutrient.

External sources of phosphorus such as stormwater runoff, septic system effluents, fertilizers, pet wastes, waterfowl, agriculture, and even rainfall can contribute phosphorus to water bodies. Also, phosphorus-enriched sediments can release phosphorus to the water through a process known as internal loading. When sediments are contributing phosphorus to a lake for example, managers can use nutrient inactivation techniques to remove phosphorus from the water column and to retard its release from the sediments.

Managers use aluminum, iron, or calcium salts for phosphorus inactivation of lake sediments. Aluminum sulfate (alum) is the most commonly used nutrient inactivation chemical for lake projects. Managers also apply alum in small doses to precipitate water column phosphorus.

Mode of action

When applied to water, alum forms an aluminum hydroxide precipitate called a floc. As the floc settles, it removes phosphorus and particulates (including algae) from the water column. The floc settles on the sediment where it forms a layer that acts as barrier to phosphorus. As sediments release phosphorus, it combines with the alum and is not released into the water to fuel algae blooms. Algal levels decline after alum treatment because alum addition reduces phosphorus levels in the water.

Nutrient inactivation is only appropriate where internal loading is a significant phosphorus source. If most phosphorus comes through external sources, alum treatment will not be effective. For appropriate nutrient inactivation projects, the length of treatment effectiveness varies with the amount of alum applied and the depth of the lake. Alum treatment in shallow lakes for phosphorus inactivation may last for eight or more years. In deeper lakes, alum treatment may last far longer.

Comparative analysis

One algaecide and eleven herbicides are currently registered by the USEPA to control algae and invasive plant species in aquatic environments.

While algaecides are not expected to affect plants, copper, endothall and aquashade (i.e. erioglaucine/ tartrazine dyes) affect both plants and algae. Four of these herbicides (i.e. carfentrazone-ethyl, copper, diquat, and endothall) and algaecides (a.i. hydrogen peroxide) are contact pesticides and work at the site of absorption. One herbicide/ algaecide, aquashade, works by inhibiting photosynthesis by shading. The remaining herbicides (i.e. 2,4-D, Glyphosate, Imazapyr, Penoxulan and Trichlopyr) are slower-acting systemic herbicides that are translocated more readily throughout the plant. Some of these products are only for use on emergent plants, others only on submersed plants, and some are selective for certain groups of plants. A comparison of the most popular aquatic herbicides is offered by Madsen (2000) and is reproduced below (Table 2).

Due to copper's potential impacts to non-target species and tendency to accumulate in ecosystems, copper should be considered as a last resource for AIS control purposes.

Most success seems to have been achieved in long-term control efforts of free floating invasive aquatic plants. A good example of successful chemical control of AIS is the maintenance of low levels of waterhyacinth in public waters of the State of Florida. While eradication has proven elusive, this program initiated in the 1970's prevents the expansion of waterhyacinth through a combination of continuous scouting and spraying of isolated colonies with the herbicides 2,4-D, diquat and glyphosate (Netherland et al. 2005).

Currently, USEPA-registered aquatic herbicides are primarily intended for applications in freshwater environments. The use of chemicals to control seaweeds has been extremely limited in the US and in other countries (Anderson 2007). Only two active ingredients (i.e. glyphosate and imazapyr) are registered by the USEPA for use in brackish and marine waters, respectively. The registration of these products has been prompted mainly by the need to control invasive *Spartina alterniflora* in wetlands of the west coast of the US. Both glyphosate and imazapyr are systemic herbicides with very specific modes of action which affect specific plant physiological process, thus increasing the treatment safety in terms of risks to human health and other non-target species. In addition, available data suggests that these products promptly degrade upon contact with water into non-toxic and non-persistent byproducts. The importance of their registration for applications to marine and transitional environments expands beyond the management of *Spartina* sp.. There are many potential applications for glyphosate and imazapyr to control marine invasive plants, but it is important to highlight that these require in depth preliminary consideration and testing. It is remarkable how little research has been done to test the suitability of available pesticides on seaweeds. In fact, our review revealed that only a few seaweeds (i.e. *Caulerpa taxifolia*, *Ecklonia radiata*, *Undaria pinnatifida*, *Sargassum muticum*), seagrasses (i.e. *Spartina alterniflora*, *Spartina anglica*), and mangrove tree (i.e. *Rhizophora mangle*) have been tested for their vulnerability to a limited number of herbicides. The results of these tests, along with the effectiveness of herbicides for the control of freshwater species can be found in tables 3 and 4.

Table 2. Use suggestions for USEPA-approved aquatic herbicides.

Source: (Madsen 2000)

Compound	Exposure Time (Water)	Advantages	Disadvantages	Systems where used effectively	Plant species response
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Copper	Intermediate (18-72 hrs)	Inexpensive, rapid action, approved for drinking water	Does not biodegrade, but biologically inactive in sediments	Lakes as algaecide, herbicide in higher exchange areas	Broad-spectrum, acts in 7-10 days or up to 4-6 weeks
2,4-D	Intermediate (18-72 hrs)	Inexpensive, systemic	Public perception	Lakes and slow-flow areas, purple loosestrife	Selective to broad-leaves, acts in 5-7 days up to 2 weeks
Diquat	Short (12-36 hrs)	Rapid action, limited drift	Does not affect underground portions; somewhat toxic to fish; persistent	Shoreline, localized treatments, higher exchange rate areas	Broad-spectrum, acts in 7 days
Endothall	Short (12-36 hrs)	Rapid action, limited drift	Does not affect underground portions	Same as Diquat	Broad spectrum, acts in 7-14 days
Fluridone	Very long (30-60 days)	Very low dosage required, few label restrictions, systemic	Very long contact period	Small lakes, slow flowing systems	Broad spectrum, acts in 30-90 days
Glyphosate	Intermediate?	Widely used, few label restrictions, systemic	No submersed control	Wetlands; Nature preserves and refuges	Broad spectrum, acts in 7-10 days, up to 4 weeks
Triclopyr	Intermediate (12-60 hours)	Selective, systemic	Restricted use	Lakes and slow-flow areas	Selective to broad-leaves, acts in 5-7 days, up to 2 weeks

At least one case of successful rapid response and subsequent eradication of invasive seaweed exists: *Caulerpa taxifolia* was eliminated from Agua Hedionda Lagoon, in California, in 2004. This eradication project is well documented in the literature (Williams and Schroeder 2004; Anderson 2005; Anderson, Tan et al. 2005) and consisted of the injection of liquid sodium hypochlorite (ca. 12% stock solution) and solid chlorine-

releasing tablets into a containment structure constructed with small polyvinyl chloride (PVC) frames covered with black 20 mil PVC sheeting.

In terms of long-term control of an aquatic invasive plants and algae in the marine environment, the most prominent example is that of seagrass *Spartina alterniflora* in Willapa Bay, Washington State and in the San Francisco Estuary, California. There, as mentioned earlier, managers have been combining glyphosate and imazapyr with other non-chemical methods, such as mowing and manual removal.

In Hawaii, unpublished preliminary results from experiments with glyphosate and imazapyr against invasive mangrove trees *Rhizophora mangle* (Kobsa, Messick et al. 2009) seem promising, and warrant further research to ensure efficiency levels and long-term risks to human health and the environment. The importance of exploring the suitability of non-registered pesticides for specific AIS control efforts is made evident by the many projects that seek special permits such as the FIFRA Section 24(c) special local need registration and the FIFRA Section 18 emergency exemption label.

Finally, the broad disinfecting properties of chlorine compounds that act as both an algaecide and molluscicide, confirms it as the chief chemical for decontamination of vessel hulls. Exploring the potential to combine chlorine with containment methods (e.g. IMProtector), neutralization products (e.g. sodium thiosulfate), and other non-chemical methods (e.g. hot water spraying) for the combat of hull fouling is timely, especially when one considers the urgent need for a protocol to be used in emergency response situations, such as in the case of removing a grounded vessel with fouling on its hull.

When compared to chlorine (gas or bleach), chlorine dioxide (ClO_2) is clearly superior. While the use of chlorine dioxide for AIS control can be costly because it must be manufactured on-site, it does not react with organic material, thus maintaining its bactericidal and inactivation effects within a wider pH range and avoiding the formation of the toxic chlorine byproducts trihalomethanes and chloramines.

Table 3. Effectiveness of USEPA-registered herbicides against some AIS found in Hawaii

Aquatic weeds recorded in HI	Common name	2,4-D	Chlorine	Diquat	Endothall	Fluridone	Glyphosate	Imazapyr	Triclopyr
Marine									
Rhizophora mangle	red mangrove						G	G	G
Freshwater (floating)									
Salvinia molesta	giant Salvinia			E		E	G		
Lemna aequinoctialis	Duckweed								
Utricularia spp.	Bladderwort	P/G		E/G		E/G			
Eichhorcia crassipes	water hyacinth	E		E		E	G	E	E
Freshwater (submersed)									
Egeria spp.	Egeria	P		G/E	F/E	E/G	P		
Potamogeton spp.	Potamogeton	P		G	E	E	P		
Freshwater (emergent)									
Sagittaria spp.	Arrowhead	E		G	G	E	E/G	E	
Thypha spp.	Cattails	G		G	P	F	E	E	
Carex spp.	sedges	F		F	P	P	G	E	
Polygonum hydropiperoides	Smartweed	E		F		F	E		E
Panicum repens	Torpedograss	P		P		F	G	G	
Ludwigia spp.	water primrose	E		F/E		F	E	E	E
Salix nigra	Willows	E		F	P	P	E	E	E
Hydrocotyle spp.	water pennywort	G		G	P	P	G	E	G

Table 4. Effectiveness of USEPA-registered Herbicides in the Control of other Aquatic Weeds

Chemical Toolbox for AIS Management in Hawaii: A Review of Substances and Methods

Weed	Copper complexes and sulfates	2,4-D	Diquat	Endothall		Fluridone	Glyphosate	Sodium Carbonate Peroxyhydrate	Triclopyr	Imazapyr
				Aquathol K Aquathol G	Hydrothol G Hydrothol 191					
Algae										
Filamentous	E	P	P		G			E		
Planktonic	E	P	G		G			E		
Branched (Chara)	E	P	G		G					
Nitella	E	P	G		G					
Floating Plants										
Bladderwort	P	P	E			E				
Watermeal	P	P	P			G	P			
Submersed Plants										
Broadleaf watermilfoil	P		E	E	E	E	P		*	
Coontail	P	G	E	E	E	E	P			
Fanwort	P	F	G	E	E	E	P			
Hydrilla	P	P	G	G	G	E	P			
Naiads	P	F	E	E	E	E	P			
Parrotfeather	P	E	E	E	E		F		*	*
Pondweeds (potamogeton)	P	P	G	E	E	E	P			
Emergent Plants										
Alders	P	E	F	P	P	P	E		*	
Alligatorweed	P	F	P	P	P	G	E		*	*
American lotus	P	E	P	P	P	F	G		*	
Buttonbush	P	E	F	P	P	P	G			
Common reed	P	P	P	P	P	P	G			*

Fragrant & white waterlily	P	E	P	P	P	E	E		*	*
Frogbit	P	E	E						*	*
Maidencane	P	P	F			F	E			
Most grasses	P	P	P	P	P	P	G			*
Pickerelweed	P	G	G			P	F		*	*
Pond edge annuals	P		G			E	E			
Rush	P	P	F	P	P	F	E			*
Slender spikerush	P		G			G	P			
Spatterdock	P	E	P	P	P	E	G-E		*	*
Southern watergrass	P	P				G	E			
Watershield	P	E	P			G	G			
Seaweeds and Seagrasses										
Caulerpa taxifolia	P		P	P	P	P				
Ecklonia radiata							P			
Sargassum muticum			P							
Undaria pinnatifida	P		P				P			
Spartina alterniflora							G			G
Spartina anglica							P			P
Adapted from http://hgic.clemson.edu/factsheets/hgic1720.htm , last updated in Dec/2008; and http://aquaplant.tamu.edu/database/index/mgmt_options.htm , http://www.hear.org/starr/hiplants/maps/ , both last updated in July/2009;										

Chapter V: Other products that have been tested for AIS control

Atrazine

Atrazine is a RUP herbicide registered for terrestrial applications for the control of pre- and post-emergence broadleaf and grassy weeds in major crops. It acts by interfering with the normal function of photosynthesis.

In situ tests on *Undaria pinnatifida* tried several methods of applying herbicides including atrazine, such as injection into the stipe or midrib, applying a gel formulation, attaching a sponge saturated with active substance to the thallus, and applying compounds inside a bag enclosing the thalli. These proved to be labor-intensive and had no appreciable impact (Sanderson 1996).

Atrazine is probably the most widely used herbicide in the world and one of the most common contaminants in ground and surface waters. Atrazine is persistent in soil and water and has been associated to various deleterious effects to human health (Kettles, Browning et al. 1997; Arbuckle, Lin et al. 2001; MacLennan, Delzell et al. 2002; Whalen, Loganathan et al. 2003; McMullin, Andersen et al. 2004; Rusiecki, Roos et al. 2004; Rayner, Enoch et al. 2005) and to the environment (Graymore, Stagnitti et al. 2001; Rohr, Sager et al. 2006), especially due to atrazine's role as a potential endocrine disrupting chemical (Hayes, Haston et al. 2003; Hayes 2004; Spanò, Tyler et al. 2004).

Azinphos-methyl

Azinphos-methyl is a highly persistent, broad spectrum insecticide. It is toxic to mites and ticks, and poisonous to snails and slugs. It is a member of the organophosphate class of chemicals. It is nonsystemic but works as both a

contact insecticide and a stomach poison. It is used primarily as a foliar application against leaf feeding insects.

Azinphos-Methyl poses potential and serious risks of concern to farm workers, pesticide applicators, and aquatic ecosystems. In 2006, EPA issued its final decision on Azinphos-methyl to phase out the remaining uses by September 30, 2012 (USEPA 2008).

Some commercial fish farmers reported informally that Guthion (Azinphos-methyl-based herbicide) is very effective for selective removal of centrarchids from bait minnow ponds, but it is regarded generally as unsuited for such use in catfish ponds. Meyer (1965) applied Guthion experimentally in ponds in Arkansas and obtained kills of green sunfish and other undesirable species without harm to channel catfish. He pointed out that water temperature and water quality have little effect on the performance of Guthion; the compound has more potential for fish control than malathion, parathion, or trithion; but Guthion-killed fish cannot be eaten (McEnnulty, Bax et al. 2001).

Benzene compounds

In laboratory experiments, benzene compounds (orthodichlorobenzene, trichloroethylene, paradichlorobenzene, trichlorobenzene, tetrachlorobenzene) have been found to be a poor control option for *Dreissena polymorpha* and *Callinectes sapidus*. Tests with *Urosalpinx cinerea*, *Eupleura caudata*, *Asterias forbesi* and *Upogebia* consisted of forming barriers with chemically treated sand to prevent larval settlement and the crossing of adults and also registered limited success. On the other hand, tests with white-tipped mud crab *Rhithropanopeus harrisi* and heavy marsh crab *Sesarma reticulatum* found benzene compounds to be successful in their control (McEnnulty, Bax et al. 2001).

Benzene, its compounds and metabolites are toxic and known carcinogens, thus their utilization for AIS control is not permitted. In fact, all pesticidal uses of benzene have been banned.

Carbaryl- organocarbamate pesticides

Carbaryl is a broad-spectrum organocarbamate that has been widely used for insect control in the terrestrial environment since DDT was banned in 1958

(USA). Concentrate active used in insecticides for poultry and pets, vegetables, fruits, forage crops. It does not bioaccumulate in the food chain and is relatively short lived in the environment (Dumbauld et al. 1997). Carbamate products are available formulated as dust, granules, liquid, pellets or wettable powder under various brand names, e.g. Nufarm, Bayer, Chemspray, Cropcare, Hortico, Yates, (Sevin -USA). Carbaryl is in use, but only until 2012.

Carbaryl is a non-persistent organocarbamate pesticide that is extremely toxic to arthropods. The chemical traditionally has been used in agriculture and forestry management to control or suppress outbreaks of insect pests (Feldman, Armstrong et al. 2000).

Since the 1960's, carbaryl has been applied to intertidal commercial beds of oysters (*Cassostrea gigas*) in Washington estuaries to control the burrowing thalassinid shrimp (*Neotrypaea californiensis* and *Upogebia pugettensis*). Burrowing shrimp reduces substrate compaction, causing oysters to sink into the substrate or to be smothered by resuspended sediment that impairs oyster growth. Carbaryl and its immediate breakdown product causes nervous system impairment resulting in behavioral changes, paralysis and death of thalassinid shrimp.

Poisoned baits were considered as one useful option for controlling *Carcinus maenas* in Washington State (Carr and Dumbauld 1999). The effects of carbaryl on non-target species have been extensively researched (Brooks 1993; Reish and Gerlinger 1997; Scaps et al. 1997; Barahona and Sanchez-Fortun 1999). Sevin (carbaryl) affected embryonic development and some had drastic effects on larval growth and survival of *Crassostrea gigas* (Davis and Hidu 1969). Larvae of the Dungeness crab (*Cancer magister*) are affected (Barahona and Sanchez-Fortun 1999; Dumbauld et al. 1997 and citations therein). Brooks (1993) found that carbaryl produced significant short-term impacts on non-target arthropods but within 51 days most populations recover to or exceed pre-spray numbers.

The effects of carbaryl on the brine shrimp, *Artemia salina* were negligible (Barahona and Sanchez-Fortun 1999). Survival of Dungeness crab larvae (*Cancer magister*) was only partially affected by carbaryl (Barahona and Sanchez-Fortun 1999; Dumbauld et al. 1997 and citations therein).

Experiments with chemicals for the control of shellfish predators such as *Asterias forbesi*, *Eupleura caudata* and *Urosalpinx cinerea* included carbamate to create barriers of chemically treated sand to exclude adults and to prevent larval settlement but registered limited success (Loosanoff et al. 1960).

Monocorophium acherusicum and *Corophium acherusicum* were particularly affected by the carbamate insecticide Sevin (Tagatz et al. 1979).

Limited effects of organophosphate and carbamate pesticides were observed on acetylcholinesterase and chlorine acetyltransferase of the polychaete, *Nereis diversicolor* (Reish and Gerlinger 1997; Scaps et al. 1997).

The pesticide is applied as a wettable powder. It slowly hydrolyses into water and eventually breaks down to carbon dioxide.

The primary disadvantage is mortality to non-target organisms due to either direct consumption of carbaryl baits or exposure to the chemical leachate. While carbaryl removes adult shrimps from oyster beds it does not prevent the reinvasion of larvae from the plankton therefore repeated treatments are required (Feldman and Armstrong 1995). There are problems with the lack of specificity and the difficulty in maintaining high concentrations in an aquatic environment of chemicals.

Carbon dioxide

Carbon dioxide has been trialed in various forms including as a gas injected into the water and as a solid pellet used in a similar way as sandblasting, both non selective methods. It has also been used selectively as a solid deployed by divers placed in contact with macroalgal thalli. *Caulerpa taxifolia*

Dry ice damaged *Caulerpa taxifolia* in an aquarium experiment, however in the field only sublethal necroses were caused Boudouresque et al. (1996).

Carbon dioxide pellet blasting has been used in a manner similar to sandblasting in water conduits to remove mussel fouling but showed limited success (Morton 1977; Boelman et al. 1997).

Preliminary results from experiments in Victoria have found that placing the mussel ropes in sealed containers of seawater injected with elevated carbon dioxide levels appears to be successful in killing the epibiota without harming the mussels (McEnnulty, Bax et al. 2001).

Chlordane

Chlordane was used as a pesticide in the United States from 1948 to 1988. In 1988, all approved uses of chlordane in the United States were canceled

(ATSDR 1994; USEPA 1997). Chlordane was available as emulsifiable concentrate or dispersible liquid and application was by agricultural spray.

The acute (short-term) effects of chlordane in humans consist of gastrointestinal distress and neurological symptoms, such as tremors and convulsions. Chronic (long-term) inhalation exposure of humans to chlordane results in effects on the nervous system. An occupational study reported an association between chlordane exposure and non-Hodgkins's lymphoma, while other human studies did not show an association between chlordane exposure and leukemia or multiple myeloma. Animal studies have reported liver cancer in mice and male rats exposed to chlordane via ingestion. EPA has classified chlordane as a probable human carcinogen (USEPA 1997).

Chlordane appears to provide some protection against boring isopods *Limnoria tripunctata* but not against *Sphaeroma* spp. (Craag, Pitman et al. 1999).

Chlorothalonil

Chlorothalonil is a broad spectrum, non-systemic GUP fungicide, mildewicide, bactericide, microbiocide, algacide, insecticide and acaricide. The exact mechanism of its mode of action is not known. It is registered for terrestrial applications only; these include food, feed and non-food crops (e.g. coffee, cucurbits, celery, forage grasses, hay, Christmas trees, ornamental herbaceous plants and turf). Chlorothalonil used to be part of the composition of anti-fouling paints, but this type of use has been discontinued by the USEPA at the time of chlorothalonil's reregistration in 1999 (USEPA 1999).

Wooden pylons used in construction of port structures used to be impregnated with chlorothalonil (alone or in combination with the insecticide chlorpyrifos) to prevent destruction of the timber by boring isopods (*Limnoria* spp.)(Craag, Pitman et al. 1999).

In situ tests, tried several methods for applying the end-use product Nopocicide (96% tetrachloroisophthalonitrile and chlorothalonil) to *Undaria pinnatifida*, such as injection into the stipe or midrib, applying a gel formulation, attaching a sponge saturated with active substance to the thallus, and applying compounds inside a bag enclosing the thalli. However, this effort was proved to be labor-intensive and to have no appreciable impact(Sanderson 1996).

Chlorothalonil is moderately persistent. In aerobic soils, the half-life is from 1 to 3 months. Increased soil moisture or temperature increases chlorothalonil decomposition. It is not degraded by sunlight on the soil surface. Chlorothalonil has high binding and low mobility in silty loam and silty clay loam soils, and has low binding and moderate mobility in sand. Chlorothalonil was not found in any of 560 groundwater samples collected from 556 U.S. sites. In very basic water (pH 9.0), about 65% of the chlorothalonil was degraded into two major metabolites after 10 weeks (EXTOXNET 1995).

Chlorothalonil is practically nontoxic to birds. Chlorothalonil and its metabolites are highly toxic to fish, aquatic invertebrates, and marine organisms. Fish, such as rainbow trout, bluegill, and channel catfish are noticeably affected even when chlorothalonil levels are low (less than 1 mgL⁻¹). The LC50 is 0.25 mgL⁻¹ in rainbow trout, 0.3 mgL⁻¹ in bluegills, and 0.43 mgL⁻¹ in channel catfish. Chlorothalonil does not store in fatty tissues and is rapidly excreted from the body. Its bioaccumulation factor is quite low (EXTOXNET 1995).

Chlorpyrifos

Chlorpyrifos is an organophosphate GUP insecticide, acaricide and miticide used to control foliage and soil-borne insect pests on a variety of food and feed crops. Application of chlorpyrifos poses acute and reproductive risks to many non-target aquatic and terrestrial animals for all outdoor uses (USEPA 2002). Its mode of action is believed to be through biotransformation by hepatic microsomal P450 isoforms via oxidative desulfuration to its oxygen analog, chlorpyrifos oxon, which subsequently inhibits acetylcholinesterase (AChE) in the nervous system (Kim, Yoon et al. 2004).

Chlorpyrifos used to be applied for the control boring isopods such as *Limnoria* spp. through treatment of wooden pylons (Craag et al. 1999 and citations therein).

A 42-d flow-through experiment was conducted to evaluate the effects of the organo-phosphate pesticide, chlorpyrifos, and microcosm size (small: 144 cm²; large: 400 cm²) on benthic estuarine macroinvertebrate colonization. Average density of the polychaete, *Neanthes succinea*, the amphipod, *Corophium acherusicum*, the barnacle, *Balanus* sp., and *Ensis minor* decreased significantly with increasing chlorpyrifos concentration from controls (326.8), to low (123.8) and high (78.8) treatments. The density decrease was significantly related only to *C. acherusicum* whose densities decreased from controls (285.8) to low (88.5) and high (43.9) dosed

microcosms. Results confirm earlier work that intrinsic design factors influence benthic macroinvertebrate community structure and determine taxa available to pesticide exposure in microcosms rate colonization of soft estuarine sediments (Flemer, Ruth et al. 1997).

Chlorpyrifos is moderately persistent in soils (half-life 60 -120 days), but can range from 2 weeks to over 1 year, depending on the soil type, climate, and other conditions. Adsorbed chlorpyrifos is subject to degradation by UV light, chemical hydrolysis and by soil microbes. Chlorpyrifos adsorbs strongly to soil particles and it is not readily soluble in water. It is therefore immobile in soils and unlikely to leach or to contaminate groundwater. TCP, the principal metabolite of chlorpyrifos, adsorbs weakly to soil particles and appears to be moderately mobile and persistent in soils. The concentration and persistence of chlorpyrifos in water will vary depending on the type of formulation. Volatilization is probably the primary route of loss of chlorpyrifos from water (half-lives of 3.5- 20 days). The photolysis half-life of chlorpyrifos is 3 to 4 weeks during midsummer in the U.S. Chlorpyrifos is very highly toxic to freshwater fish, aquatic invertebrates and estuarine and marine organisms. Application of concentrations as low as 0.01 pounds of active ingredient per acre may cause fish and aquatic invertebrate deaths. Chlorpyrifos accumulates in the tissues of aquatic organisms. Studies involving continuous exposure of fish during the embryonic through fry stages have shown bioconcentration values of 58 to 5100. Due to its high acute toxicity and its persistence in sediments, chlorpyrifos may represent a hazard to sea bottom dwellers. Smaller organisms appear to be more sensitive than larger ones (EXTOXNET 1995; USEPA 2002).

Croton seed powder

Croton seed powder is the residue after croton oil is extracted from the seed of various species of the *Croton* genus, including *Croton tiglium*, *Croton camaza*, *Croton glandulosum*, *Croton muricatum*, and *Croton officinale*. The powder is a traditional fish poison in China where it has been used to eliminate predators from carp nursery ponds. The ripe pounded fruit is used in Java and by the Dayaks in Borneo to poison fish. In the Philippines, the fruit or crushed leaves of this species are used in poisoning fish. When the seeds are used for this purpose they are pulverized and put in sacks, which are placed in ponds or rivers. This is an erect or more or less spreading shrub or very small tree. Roots contain tannin at 65 percent (Lennon, Hunn et al. 1971).

Dalapon

Dalapon is an organochlorine herbicide and plant growth regulator used to control specific annual and perennial grasses, such as quackgrass, Bermuda grass, Johnson grass, as well as cattails and rushes. It is classified by the USEPA as a General Use Pesticide (GUP)(EXTOXNET 1995).

Dalapon has the potential to cause the following effects from a lifetime exposure at levels above the MCL: increased kidney-to-body weight.

Dalapon (2,2-Dichloropropionic Acid) was experimentally applied for *Spartina* spp. control in the United Kingdom using an Agrocatt 8-wheel, low ground pressure vehicle fitted with agriculture spraying equipment (Evans 1986). Dalapon reportedly caused 80% mortality, but was generally considered to be ineffective (Eno et al. 1997). Dalapon is released directly to the environment in its use as an herbicide for the control of annual and perennial grasses. In Northern Ireland estuaries, dalapon had been used since the 1960's to control *Spartina anglica*, but the method has been discontinued (Hammond and Cooper 2002).

Trade names for Dalapon include Revenge, Alatex, and Basfapon.

DDT (Dichloro-diphenyl-trichloroethane)

DDT was first synthesized in 1874, but its insecticidal properties were not discovered until 1939. It was used during the second half of World War II to control mosquitoes spreading malaria and lice transmitting typhus among civilians and troops, resulting in dramatic reductions in the incidence of both diseases. After the war, DDT was made available for use as an agricultural insecticide. In 1962, American biologist Rachel Carson published *Silent Spring*, a book that exposed the environmental impacts of the indiscriminate spraying of DDT in the US and questioned the logic of releasing large amounts of chemicals into the environment without fully understanding their effects on ecology or human health. DDT was subsequently banned for agricultural use worldwide under the Stockholm Convention, but its limited use in disease vector control in other countries continues to this day and remains controversial (EXTOXNET 1995).

DDT affected embryonic development and some had drastic effects on larval growth, shell configuration and survival of *Crassostrea gigas* (Davis and Hidu

1969) and was very toxic to *Monocorophium insidiosum* (Reish 1993). Environmentally derived immunomodulation in *Mytilus edulis* mussels from the Venice lagoon in response to DDT was examined by Pipe et al. (1995).

Dichlobenil

Dichlobenil (2,6-dichlorobenzonitrile) is a herbicide used to control weeds, and grasses in agricultural, residential and industrial areas. Dichlobenil used to be applied in aquatic sites for the control of freshwater broad-leaved emergents, most submerged and also rooted floating weeds including Water-milfoil (*Myriophyllum* spp) Curled Pondweed *Potamogeton crispus*, Fennel Pondweed (*Potamogeton pectinatus*), Water-Crowfoot *Ranunculus* spp, and others (Hofstra and Clayton; Walker 1964), but this utilization has been discontinued- dichlobenil herbicides are not registered for aquatic applications in the US (USEPA 1998). The application of dichlobenil to aquatic environments has recently been discontinued in the UK as well. The final product use-by date in that country is 18 March 2010 (Vassiliou 2008).

In situ tests, tried several methods of applying herbicides including dichlobenil to *Undaria pinnatifida*, such as injection into the stipe or midrib, applying a gel formulation, attaching a sponge saturated with active substance to the thallus, and applying compounds inside a bag enclosing the thalli. Methods proved to be labor-intensive and had no appreciable impact (Sanderson 1996).

The USEPA classifies dichlobenil as of low acute toxicity to humans: slightly toxic by the oral, dermal, and inhalation routes. Dichlobenil is not an ocular or dermal irritant or a skin sensitizer. Dichlobenil's metabolite, 2, 6-dichlorobenzamide (BAM) is slightly toxic by oral route. Dichlobenil is classified as possible human carcinogen. Additional information is needed to determine the cancer classification of BAM. Dichlobenil dissipates in the environment (on soil and in surface water) principally by volatilization. However, it is persistent under field conditions that reduce the potential for volatilization (i.e., cooler climates). When transformation proceeds through aerobic soil metabolism, the metabolite, BAM is generated (13.1% at 50 weeks). Under conditions where dichlobenil does not volatilize there is potential for both dichlobenil and BAM to move to ground water in coarse-textured soils low in organic matter. Both dichlobenil and BAM can be extremely mobile and persistent under anaerobic conditions (USEPA 1998).

On an acute basis, dichlobenil is practically nontoxic to birds, mammals, honey bees; slightly to moderately toxic to aquatic invertebrates and estuarine

organisms; and moderately toxic to fish. Dichlobenil is practically nontoxic to birds on a subacute dietary basis, but insufficient data are available to assess chronic avian toxicity. Dichlobenil is toxic to non-target terrestrial and aquatic plants. Dichlobenil may chronically affect fish at levels as low as 0.33 ppm and may chronically affect aquatic invertebrates at levels as low as 0.75 ppm. The dichlobenil degradate, BAM is slightly toxic to mammals and practically nontoxic to fish and aquatic invertebrates on an acute basis (USEPA 1998).

Dieldrin/ Aldrin

Dieldrin and aldrin are synthetic organochlorine pesticides that act as effective contact and stomach poisons for insects. Originally, they were used as broad-spectrum soil insecticides for the protection of various food crops, as seed dressings, to control infestations of pests like ants and termites, and to control several insect vectors of disease. In 1972, the USEPA cancelled all but three specific uses of these compounds (subsurface termite control, dipping of non-food plant roots and tops, and completely contained moth-proofing in manufacturing processes), which by 1987 were voluntarily cancelled by the manufacturer. Dieldrin is an extremely persistent organic pollutant and tends to bioaccumulate in the food web. Long-term exposure to dieldrin has proven toxic to humans and other animals and has been linked to human health issues including Parkinson's disease, breast cancer, and immune, reproductive, and nervous system damage (USEPA 2003).

Dieldrin affected embryonic development and some had drastic effects on larval growth and survival of *Crassostrea gigas* and seems to have provided some protection against *Limnoria* sp. but not *Sphaeroma* sp. (Craag, Pitman et al. 1999; McEnnulty, Bax et al. 2001).

Endosulfan

Endosulfan is a broad spectrum contact insecticide and acaricide registered by the USEPA for use on a wide variety of vegetables, fruits, cereal grains, and cotton, as well as ornamental shrubs, trees, vines, and ornamentals for use in commercial agricultural settings (USEPA 2002). Due to its acute toxicity, potential for bioaccumulation, and role as an endocrine disruptor, endosulfan has been banned or significantly restricted or banned in several countries, but not in the US.

Endosulfan was considered as a potential fish control chemical (Lennon 1970). The toxicology of Thiodan (an endosulfan-based product) in fish and aquatic invertebrates was studied by Schoettger (1970) to determine potentials of the compound as a fish toxicant. He found that fish were at least seven times more susceptible than invertebrates to Thiodan; exposures of 2 hours to 50 mgL⁻¹ (ppm) of Thiodan were not toxic to fertilized eggs of rainbow trout; Thiodan has little value as a selective toxicant against carp and suckers; the compound may be a good general fish toxicant under certain conditions; and residues of the toxicant occur in the skin and muscles of fish exposed to acute and multiple subacute concentrations.

Monocorophium acherusicum *Corophium acherusicum* dominated species abundance in sediments fortified with fenvalerate and endosulfan pesticides (Flemer, Stanley et al. 1995). Endosulphan inhibited heart rate and caused respiratory toxicity in *Mytilopsis sallei* in Indian experiments, however concentrations that caused mortality were not presented (McEnnulty, Bax et al. 2001).

Endrin

Endrin was reported by Henderson, Pickering, and Tarzwell (1959) to be by far the most toxic of the insecticides to all species of fish and the most toxic chemical that had been tested in their laboratory. The most extensive use of endrin as a fish toxicant appears to have been in Malaya where Soong and Merican (1958) removed all fish from 108 mining pools and fish ponds prior to restocking. Iyatomi et al. (1958) experimented with three formulations of endrin against fish in paddyfields in Japan and found that toxicity may persist for more than a month. Treatment of a small lake in Michigan with 0.008 mgL⁻¹ (ppm) was only partially successful, and further use of endrin was not recommended (Hooper et al., 1964). More recently, Bhimachar and Tripathi (1967) reported that endrin at 0.1 mgL⁻¹ (ppm) has been used to kill predatory and weed fishes in carp nursery ponds in India. Large amounts of charcoal served well to detoxify the treated water. (Lennon, Hunn et al. 1971).

Endrin affected embryonic development and some had drastic effects on larval growth and survival of *Crassostrea gigas* (Davis and Hidu 1969).

Endrin appears to provide some protection against *Limnoria tripunctata* but not *Sphaeroma* (Craag et al. 1999).

Fluazifop-P-Butyl

Fluazifop-P-Butyl is the active ingredient in Fusilade, a post-emergence selective herbicide for perennial, annual grass weeds (over-the-top in cotton and soybean).

Spartina infestations in the intertidal Fluazifop-P-Butyl is usually mixed with a adjuvant (surfactant of crop oil) and applied with a pressurized backpack sprayer 1:100 at 1000 liter/ha, as soon as exposed by lowering tide (RGAG 1998).

Ichthyothereol

Ichthyothereol is a substance extracted from plants of the genus *Ichthyothere* and from certain species of the genus *Clibadium*. Both genera are found in parts of South and Central America. Ichthyothereol is a polyacetylene compound is reported to be so toxic to fish that they will actually “jump out of the water” if *Ichthyothere terminalis* leaves are used as bait (Cascon, Mors et al. 1965).

Native peoples in the Lower Amazon Basin in Brazil have used the leaves of this herb as a fish poison for a long time (Cascon, Mors et al. 1965) and some South American indigenous peoples also use the leaves of *Clibadium sylvestre* as a fish toxicant (Lennon, Hunn et al. 1971).

The active ingredients in the herb leaves are ichthyothereol and ichthyothereol acetate. Cascon et al. (1965) reported on the isolation and identification of these compounds. These authors stated that guppy reacts promptly to minute quantities of the poison with extreme agitation and dies after a few minutes. Simple aqueous extracts of the leaves were rapidly and extremely toxic to guppy and goldfish in laboratory tests as well (Quilliam and Stables 1968). The typical response of fish was violent activity, followed by loss of coordination, paralysis, and death.

Malathion

Malathion is an organophosphate insecticide that has been registered for use in the United States since 1956. It is used in agriculture, residential gardens, public recreation areas, and in public health pest control programs.

There is extensive literature on the biological activity of malathion, including its effects on fish and aquatic invertebrates (Walker, 1969). There is a 1,000-fold range in toxicity from a few parts per billion to several parts per million between fish species depending on exposure, temperature, pH, and water hardness. Some private fish farmers make use of this differential toxicity to control predaceous or competitor fishes in production ponds. Al-Hamed (1967) found, for example, that wild fishes could be eliminated from ponds in Iraq without harming the cultured carp. Undesirable sunfishes can be removed selectively from minnow ponds by applying 0.5 mgL⁻¹ (ppm) of malathion when water temperatures are between 4.4 and 26.7° C (U.S. Bureau of Sport Fisheries and Wildlife, 1970b). The possibility remains that malathion may have some registerable uses in fish culture if residue tolerances in water and fishery products are established (Lennon, Hunn et al. 1971).

Metaldehyde

Metaldehyde is a molluscicide used to control snails and slugs on a wide variety of terrestrial sites, including turf, ornamentals, berries, citrus, and vegetables (USEPA 2006).

Metaldehyde is highly mobile in soils, and is generally stable to abiotic degradation mechanisms such as hydrolysis and photolysis. Metaldehyde is primarily dissipated from soils through biodegradation under aerobic conditions, with a half-life of approximately 2 months. Under anaerobic conditions, the half-life of metaldehyde is much higher (>200 days). Its low vapor pressure and Henry's Law constant indicate that volatilization from soils and water surfaces will not be an important transport process. In addition, the results of a laboratory volatility study suggest that volatilization losses from soil surfaces will be minor. Acetaldehyde is the primary decomposition product of metaldehyde. Acetaldehyde is a relatively short-lived metabolite in the environment, and is readily oxidized to acetic acid and ultimately to carbon dioxide and water (USEPA 2006).

The results of acute toxicity studies with a surrogate freshwater fish (rainbow trout) show that metaldehyde is slightly toxic to freshwater fish on an acute basis. No acute toxicity data with estuarine/marine fish are available for metaldehyde. In an acute toxicity study for a freshwater invertebrate (*Daphnia magna*), no treatment-related effects were observed at the highest concentration of metaldehyde tested. No acute toxicity data with estuarine/marine invertebrates are available for metaldehyde. No chronic

toxicity data are available for metaldehyde in freshwater fish and invertebrates. No toxicity data are available for acute or chronic exposures to estuarine/marine fish or invertebrates, or aquatic plants (USEPA 2006).

According to label directions, metaldehyde is not to be applied directly to water, to areas where surface water is present, or to intertidal areas below the mean high water mark, as drift and runoff may be hazardous to aquatic organisms in water adjacent to treated areas (USEPA 2006).

Poychlorpinene

Research on fish toxicants in Russia resulted in the development of polychlorpinene (Burmakin, 1965). The compound is a chlorinated turpentine, resembling toxaphene in some respects. By 1963, 118 lakes in Russia had been treated with the compound at 0.05 to 0.20 mgL⁻¹ (ppm) to control rough fish, and some tests had been initiated in small lakes in Germany (Schäperclaus, 1963). By 1965, 241 lakes in Russia, totaling 17,000 ha (42,000 a), had been reclaimed with polychlorpinene (Burmakin, 1967). The toxicant persisted up to 1.5 years in lakes in northern Russia, and degradation in water depends on concentration, water temperature, alkalinity, depth, and the extent of water mixing. Small, shallow lakes are preferred for treatment, and intensive management for production of food fishes usually follows the reclamations.

Bizyaev, Antimov, and Moskalev (1965) noted that the search for fish toxicants is continuing in Russia. They added that polychlorpinene has disadvantages such as non-specificity to fish, long persistence in water, and safety problems when used near human populations. In contrast with polychlorpinene, the investigators are seeking toxicants that are highly toxic to fish, harmless to warm-blooded animals, and quickly degradable (Lennon, Hunn et al. 1971).

Formaldehyde/ formalin

Formalin is a 10% aqueous solution of formaldehyde. It is used as a disinfectant, fungicide and germicide.

Injection of poisons including formalin using pole spears to control crown-of-thorns starfish *Acanthaster planci* was found to be locally effective on the

Great Barrier Reef. Kill rates were close to 100% depending on the poison used, maximum rate of 140 injections per hour (Birkeland and Lucas 1990; Thresher et al. 1998).

Limited success was observed by Edwards et al. (2000) in the prevention of the spread of zebra mussels during fish hatchery and aquaculture activities using formalin.

Unsuccessful attempts for the large-scale removal and destruction of *Urosalpinx cinerea* were conducted in Essex Rivers, UK, using physical and chemical control methods in the 1950s. This included chemical treatments by immersion of *Urosalpinx* and their host oysters in solutions of formalin or as chemically impregnated barriers (Hancock 1959; Spencer 1992).

Glufosinate-ammonium

Glufosinate-ammonium is a non-selective foliar herbicide that is used for pre-plant and post-emergent control of broadleaf weeds. It acts by inhibiting glutamine synthesis, which leads to poisoning in plants via the overproduction of ammonia. It is registered for use on the food crops (apples, berries, canola, etc.) and non-crop areas (e.g. golf course turf, residential lawns, ornamentals) or its ammonium salt DL-phosphinotricin is an active ingredient in several nonselective systemic herbicides. End-use products include Basta, Rely, Finale, Challenge and Liberty. It interferes with the biosynthetic pathway of the amino acid glutamine and with ammonia detoxification.

It was successfully used in Portugal for the control of freshwater vegetation water hyacinth (*Eichhornia crassipes*) (Moreira et al. 1999).

Hydrochloric acid

The injection of poisons, including hydrochloric acid, using pole spears was found to be locally effective on the Great Barrier Reef to control crown-of-thorns starfish *Acanthaster planci*. Kill rates were close to 100% depending on the poison used, maximum rate of 140 injections per hour (Birkeland and Lucas 1990).

Lindane

Lindane is an organochlorine insecticide. It affected embryonic development and some had drastic effects on larval growth and survival of *Crassostrea gigas* (Davis and Hidu 1969). Lindane has been used to control boring isopods, *Limnoria* spp. but not *Sphaeroma* (Craag et al. 1999).

Environmentally derived immunomodulation exposed to lindane has been examined in mussels *Mytilus edulis* Successful. from the Venice lagoon (Pipe et al. 1995).

Pentachlorophenol (PCP)

PCP is no longer used as a molluscicide because of potential harmful effects on handlers and the environment (Coglianese and Neff 1982; Perrett and Whitfield 1996).

PCP is highly toxic to most living organisms although marine and freshwater crustaceans *Callinectes sapidus* appear to be moderately tolerant of PCP and its sodium salt (NaPCP) (Coglianese and Neff 1982).

Sodium salts of PCP affected embryonic development and some had drastic effects on larval growth and survival of *Crassostrea gigas* (Davis and Hidu 1969).

Santobrite a sodium pentachlorophenate preparation was trialed to control of *Cyprinus carpio* in Victorian waters but proved to be expensive and rarely effective (Barnham 1998a, 1998b). *Dreissena polymorpha* Successful. Freshwater. Pentachlorophenate has been used as a molluscicide against snails for human schistosomiasis control and has been used to control *Dreissena polymorpha* (Perrett and Whitfield 1996).

The LC50 of sodium pentachlorophenate to fingerling channel catfish is 0.46 mgL⁻¹ (ppm) (Clemens and Sneed, 1959). Walker (1969) stated that concentrations as low as 0.06 mgL⁻¹ (ppm) are lethal to fish under laboratory conditions and that piscicidal activity varies with temperature, pH, and other factors. A private fish farmer informed us that sodium pentachlorophenate effectively removed fish from his ponds, but did not kill tadpoles or snails. He discontinued use of the compound upon discovery that its residues were detrimental to fish during early developmental stages, causing excessive mortalities and teratogenesis, especially in goldfish.

Thanite

Thanite was tested at 0.7 to 1.5 mgL⁻¹ (ppm) in ponds in Illinois to determine its potential for live removal of fish and for selective and total kills of fish (Lewis, 1968). The compound at first has an anesthetizing effect on fish, allowing desirable species to be collected easily at the surface during this stage. Their recovery in fresh water is rapid and complete. The live removal of adult largemouth bass from the ponds was highly successful. Lewis (1968) also observed that Thanite can be applied as a selective toxicant against centrarchids in the presence of cyprinids and ictalurids. Working in conjunction with Lewis, Leland (1964) demonstrated that the blood of fish killed by Thanite contains cyanide, but that there is a rapid loss of cyanide from the blood of exposed fish held in fresh water.

Additional studies on Thanite as a fishery tool are in progress at the Fish Control Laboratories, U.S. Bureau of Sport Fisheries and Wildlife, La Crosse, Wisconsin and Warm Springs, Georgia. (Lennon, Hunn et al. 1971)

Sodium cyanide

The use of sodium cyanide as a fish toxicant was introduced by Bridges (1958) as a result of his experiments in laboratory aquaria and farm ponds in Illinois. He reported that 1 mgL⁻¹ (ppm) of sodium cyanide, easily applied as 28-gram Cyaneggs, kills various species of warm-water fishes in a variety of temperature and pH conditions at a cost of 0.55 US dollars/1,000m³. Within minutes, fish begin coming to the surface, and desirable species can be removed to fresh water for complete recovery. Water in small farm ponds remained toxic for about 4 days.

Lewis and Tarrant (1960) continued experiments in Illinois to demonstrate the effectiveness of sodium cyanide as a collecting tool and general toxicant. They recommended the compound for preparing rearing and brood ponds. In relation to these experiments, Leland (1964) studied the loss of cyanide from the water, soil, and fishes. He determined that residues of cyanide are no problem in bottom muds; the toxicant disappears from water within 4 to 20 days, with dissipation slower in cold waters or under ice; and cyanide residues in live fish drop to less than 0.1 mg/kg after 24 hours in fresh water.

Multiple uses of sodium cyanide were exhibited in Nebraska by Miller and Madsen (1964) including the live removal of northern pike from nursery ponds, the salvage of fish from irrigation canals, the sampling of fish populations in lakes and streams, and the eradication of undesirable fish from lakes. The toxicant was used with variable success in South Dakota for simultaneous removal of live walleyes from rearing ponds and reclamation of the ponds for new stocking in the following year (Hanten, 1966). Whitley (1967) reviewed the effects of sodium cyanide on fish in Missouri, noting that the compound is toxic at all temperatures, but more rapidly so in warm water. At present, the major use of cyanide is centered in the lower Mississippi River Valley where fish farmers apply many thousands of kilograms in fish cultural ponds to eliminate competing fishes and predaceous invertebrates (Prewitt, 1970). Fish-eating snakes and birds are killed sometimes by eating fish treated with high concentrations (10 mgL^{-1} [ppm] or more) of cyanide (Lennon, Hunn et al. 1971).

Sodium sulfite

Westman and Hunter (1956) made an experimental application of sodium sulfite at 168 mgL^{-1} (ppm) to a small pond in New Jersey to salvage certain fishes and reduce numbers of others. They concluded that salvage operations would be practical in small areas, but that the compound is too expensive for large waters. The sodium sulfite lowers the concentration of dissolved oxygen in the water very quickly, and fish suffocate. The water is non-toxic, and the dissolved oxygen is restored rapidly. Affected fish are salvaged by removing them to fresh water. Species with inferior mouths have difficulty gulping air at the surface, causing them to be more susceptible to suffocation.

Grice (1961) reported that 100 mgL^{-1} (ppm) of sodium sulfite followed by 50 mgL^{-1} (ppm) about 22 hours later were not effective in incapacitating fingerling walleye for removal from a pond in Massachusetts. More recently, Vanderhorst and Lewis (1969) used cobalt chloride to catalyze sodium sulfite, and concluded that the combination has promise for selective removal of fish, particularly channel catfish (Lennon, Hunn et al. 1971).

Sodium hydroxide

Pellets of sodium hydroxide have been dropped into the nests of unwanted sunfishes to kill eggs and fry (Jackson, 1956). The control is limited, however, to waters where nests can be located and treated easily with reasonable expenditure of time and effort (Lennon, Hunn et al. 1971).

Tobacco waste (Nicotine)

Tobacco wastes are added to milkfish ponds as fertilizer in Southeast Asia and have the advantage that the nicotine kills aquatic insects (Bardach, 1968). Personal communications disclose that tobacco wastes are used at about one ton per acre in ponds in Taiwan. The combination of nicotine from the tobacco and oxygen-depletion resulting from the decomposition of the plant acts to poison and suffocate unwanted fish, fish parasites, and possibly bacteria. Tobacco dust at 12 to 15 kg/ha of nicotine eliminates fish, snails, and polychaete worms. The tobacco dust also serves as a direct fertilizer and accelerates the fertilizing action of rice straw, rice bran, and other plant materials.

In India, Konar (1970) suggested that nicotine may be very useful as a fish-collecting aid and toxicant. Rohu exposed to 3.2 mgL⁻¹ (ppm) of nicotine and punti exposed to 5.0 mgL⁻¹ (ppm) surfaced within 5 to 10 minutes and recovered within 2 to 4 minutes in fresh water. Some fish remaining in the solutions of nicotine exhibited signs of acute poisoning and perished, but others showed no symptoms of poisoning. The low concentrations of nicotine tested were less toxic to aquatic insects than to the fish.

Toxaphene

Toxaphene consists of a mixture of polychloro bicyclic terpenes with a predominance of chlorinated camphene. At highest purity, it contains 67 to 69 percent of chlorine. It has been used widely against a variety of insect pests on agricultural crops (Gebhards, 1960). Surber (1948) was perhaps the first to test toxaphene against fish, and he observed that 0.04 mgL⁻¹ (ppm) of toxaphene killed all fish in a small pond. Noting that toxaphene was much more toxic than rotenone to fish, Tarzwell (1950) suggested that the compound may be useful in fish management. The first major field trials of

toxaphene as a fish toxicant were conducted by Hemphill (1954) in two Arizona lakes in 1951. A concentration of 0.1 mgL⁻¹ (ppm) eliminated the rough fish, including carp, in one lake and greatly reduced their numbers in another. He added that the killing action of toxaphene was slow in comparison with rotenone and extended over a period of days. The insect life in the lakes was severely affected, but not eliminated.

Tanner and Hayes (1955), evaluating toxaphene as a fish toxicant in Colorado, indicated that a lake may be treated effectively with the compound for about \$0.10/1,000 m³ as compared with \$0.77/1,000 m³ with rotenone. Admitting that toxaphene is attractive from the standpoint of economy, they advised that it is an extremely powerful poison of greater toxicity to warm-blooded animals than rotenone and requires greater precautions in handling. They concluded that toxaphene may persist for at least 7 months at toxic level in a lake at pH 8.0 or higher.

In Michigan, Hooper and Grzenda (1957) In Michigan, Hooper and Grzenda (1957) demonstrated that toxaphene is more toxic to fish in hard water than in soft water, and more toxic in warm water than in cold water. Although toxaphene at 0.1 mgL⁻¹ (ppm) gave good results against fish, the lakes remained toxic to fish for periods of 2 to 10 months. Bottom invertebrates are killed in large numbers, but they quickly reappear in abundance.

The observation that 5 µgL⁻¹ (ppb) of toxaphene in hard water killed small fish, but left large bluegill and largemouth bass unharmed, prompted Fukano and Hooper (1958) to suggest that the compound has potential as a selective poison. Stringer and McMynn (1958) applied the compound at 0.01 to 0.10 mgL⁻¹ (ppm) in eight alkaline lakes in British Columbia, and eliminated all fish and amphipods. They noted that toxaphene is an effective and economical fish toxicant, but the lakes were still toxic to fish 9 months after treatment. In a follow-up study, Stringer and McMynn (1960) discussed methods for dispensing toxaphene, the killing time for fish, the lower lethal concentrations for a number of fish species, and factors influencing degradation. They pointed out that small concentrations of toxaphene applied to control cyprinids and cottids in deep, clear, stratified lakes in British Columbia may persist at toxic level for 2 years. On the other hand, detoxification proceeds so rapidly in some turbid lakes that relatively high concentrations produced only partial kills of fish.

In Iowa, tests of toxaphene against fish in the laboratory and field were encouraging (Rose, 1958). Carp and bullheads required over 25 µgL⁻¹ (ppb) for kills in cold, clear water, whereas 200 µgL⁻¹ (ppb) were needed against

the same species in highly turbid water. Silt was suspected of having a direct detoxifying effect.

The results of 4 years of reclamation efforts with toxaphene in Nebraska lakes were reviewed by McCarragher and Dean (1959). They found that at least 0.5 mgL⁻¹ (ppm) of toxaphene was required for complete kills of fish in Sand Hill lakes having moderate alkalinity, high turbidity, and pH 8.5 to 9.5. They recorded serious problems, however, that arose during aerial applications of the toxicant. An aerial application of 0.61 mgL⁻¹ (ppm) of toxaphene in one lake killed every wild duck, but carp and bullheads survived. A similar application of 0.52 mgL⁻¹ (ppm) in another lake killed all fish, but also killed 33 percent of the mallards and 29 percent of the gadwalls, but less than 10 percent of the gulls and grebes present in the treated area. Each of the aerial applications of toxaphene was accompanied by losses of waterfowl ranging from 15 to 100 percent. Dead mammals possibly associated with the operations included raccoon, dog, skunk, and cow. In contrast, there were few mortalities of birds when toxaphene was sprayed on the water from a boat.

Gebhards (1960) documented the increasing use of toxaphene in states and provinces of western North America. He also discussed the toxicity of toxaphene to humans, livestock, waterfowl, fish, and aquatic invertebrates, and stated that the factors increasing the rate of detoxification of toxaphene are sunlight, high concentration of dissolved oxygen, high temperature, water circulation, and turbulence. Kallman, Cope, and Navarre (1962) demonstrated that aquatic vegetation in a treated lake accumulated high concentrations of toxaphene and that rainbow trout and black bullhead (*Ameiurus melas*) concentrated the toxicant within their bodies. Hunt and Keith (1963) discussed the biological magnification of toxaphene residues that results in death of birds. Following the treatment of Big Bear Lake in California, Johnson (1966) recommended that toxaphene not be used as a fish poison anywhere in the state. Terriere et al. (1966) observed the persistence of toxaphene in Oregon lakes up to 6 years, with residues accumulating up to 14 mgL⁻¹ (ppm) in rainbow trout and 17 mgL⁻¹ (ppm) in aquatic plants. Other reviews on the performance and persistence of toxaphene were made by Nehring (1964), Johnson, Lee, and Spyridakis (1966), Henegar (1966), and Moyle (1968).

A survey in 1966 indicated that toxaphene ranked second to rotenone as a fish toxicant in the United States, but ranked first in Canada (Stroud and Martin, 1968). The limited use of the toxicant against fish in Germany was described by Anwand (1968b). Applications of the compound as a fish toxicant declined rapidly in the United States in the late 1960's, however, due in part to a ban imposed by the U.S. Department of the Interior in 1963 (Dykstra and Lennon, 1966). This ban was prompted by the persistence of toxaphene in

water, its high toxicity to invertebrates and vertebrates, especially waterfowl, and the accumulation of residues in plants and animals. Further use of toxaphene as a fish toxicant in federal projects or federally aided projects was forbidden. Walker (1969) observed that toxaphene has been one of the most extensively misused fish toxicants in the United States and Canada.(Lennon, Hunn et al. 1971)

Simazine

Simazine is a selective pre-emergent herbicide formulated as Wettable Powder. Very high concentrations of Simazine were needed to inhibit zoospore germination of the macroalga, *Ecklonia radiata* (Burrige and Gorski 1998).

Chapter VII: Conclusions and Recommendations

This assessment was carried out to identify available and potential chemical methods for eradication and control of nuisance fish, mollusks, vegetation, algae, and biofouling organisms in marine, freshwater, and brackish environments of Hawaii. The review results indicate that, whereas some progress in the field of AIS management has occurred in the last decade or so, efficient combat of aquatic invasive species remains as a major technical, scientific, and policy challenge for environmental managers all over the world.

Unfortunately, while new aquatic invasions rapidly increase in frequency and magnitude, propelled by globalization and intensified maritime traffic, investments in the development of methods and tools to deal with these introductions have been substandard at best. As a result, few chemical products and application methods have been thoroughly tested against AIS and even fewer meet the expected needs, goals, and risks associated to some common aquatic biocontrol projects.

As pointed out by many authors (Birkeland and Lucas 1990; Bax, Carlton et al. 2001; McEnnulty, Bax et al. 2001; Bax, Hayes et al. 2002; Anderson 2007), the array of AIS management choices available for managers is significantly limited by the nature and characteristics of the environment to be treated, especially in the case of the use of chemicals. Logically, the treatment of small, closed water bodies, such as ponds and small lakes is considerably easier to carry out and usually involves fewer risks than combating AIS in larger interconnected water bodies, such as streams, rivers, or in coastal ecosystems, such as estuaries. In small closed water bodies, managers are able to better predict and limit treatment side-effects and can model and monitor the fate of the treatment residues in the environment. These tasks are extremely difficult to perform in open and interconnected water bodies. Additionally, in small closed systems managers can better understand and if necessary manipulate water quality conditions and potential synergies that affect the efficacy of the control methods. These disparities are reflected in the variety of control

options available for the control of AIS in freshwater versus the options available for the control of AIS in coastal and marine systems. Few studies have ventured into saltwater to explore control methods for marine and brackish AIS. This is field of research in its infancy and development has been slow in spite of its pressing demand.

For fish management projects, the chemical control options are currently limited to two active ingredients, rotenone and antimycin A. According to USEPA's most recent reregistration decisions, these active ingredients can only be applied into environments that have no connection with the marine environment (USEPA 2007; USEPA 2007).

Rotenone has been subjected to comprehensive testing and scrutiny, and a recently developed formulation (i.e. CFT Legumine) apparently represents a substantial improvement in the field of fish control projects, as it supposedly contains inert ingredients that are far less toxic than those in previous formulations. The production of rotenone seems to be steady and continued research is expected to clarify its toxicology even further. In contrast, research on antimycin A is incipient and its continued production is uncertain. Antimycin A is reportedly less toxic to non-target invertebrates than rotenone. However, the exploration of antimycin A as a piscicide is a relatively recent development and its long-term environmental effects are still obscure. Antimycin A has been commercialized by a single company, Aquabiotics Ltd., and its production has recently been put on a halt (Mary Romeo, pers. comm.).

Rotenone and antimycin A are both valuable tools for the control of established invasive freshwater fish species (e.g. tilapia, mosquitofish) that cause substantial impacts to Hawaiian endangered native species and ecosystems (e.g. O'pae'ula in anchialine pools and endemic waterfowl in coastal wetlands). Nevertheless, as in the case of any chemical application, rotenone and antimycin A treatments represent potential risks to non-target species. For this reason, applications should only be carried out by trained personnel following government approved protocols. These protocols should include guidance on how to conduct project-specific preliminary tests such as bioassays to measure the sensitivity of target and non-target species to a certain batch, and dispersion tests such as dye experiments, to determine rate of dispersal in different water bodies and weather conditions.

The legality of pesticide applications to waters of the state of Hawaii, including those of rotenone and antimycin A, is currently ambiguous. Until April 2011, licensed aquatic pesticide applicators are authorized under Federal law and regulations (i.e. FIFRA and the USEPA's interpretation of the CWA), to apply rotenone and antimycin A to waters of the US without an NPDES permit. If a NPDES is not required, then a CWA 401 State Certification is also not required. One state regulation (HAR 11-55) also authorizes licensed applicators to apply FIFRA-registered pesticides, such as rotenone and antimycin A to state waters. However, another state regulation (HAR 11-54) prohibits the addition of any biocide to any waters of the state. Inasmuch as the intent of the state agency in charge of administering water quality and water pollution control in Hawaii (i.e. DOH) is concerned, it is clear that when that department changed HAR 11-55 to be aligned with the USEPA's interpretation of the CWA, it meant to allow licensed applicators to use certain registered aquatic pesticides in state waters. Nevertheless, DOH's failure to also adjust HAR 11-54 to match the terms set in HAR 11-55 has created a convoluted legal situation that compromises the lawfulness of chemical control projects in Hawaii.

Consultations with the USEPA and the DOH indicate that this legal incongruity is due to be resolved sometime before April 2011. The USEPA is working with state agencies to develop General NPDES permits for certain aquatic pesticide applications. According to the timeline set by the USEPA (USEPA 2009) the DOH must propose draft permits by April 2010 and changes in HAR 11-54 and 11-55 are expected to take place before April 2011. Negotiations with DOH regarding the need for collaboration to resolve legal and regulatory issues that hinder the development of AIS management tools have been initiated. In a meeting between representatives from DAR/ DLNR (i.e. Administrator Dr. Dan Polhemus, Biologist Tony Montgomery, Ballast Water and Hull Fouling Coordinator Jason Leonard and myself) and DOH representatives (i.e. Deputy Director for Environmental Health Laurence Lau, Clean Water Branch Chief Alec Wong, and other Clean Water Branch personnel Joanna Seto and Edward Chen) the AIS problem and management needs were discussed and solutions for current legal impediments discussed. Further dialogue should be promoted and should address specific terms for the upcoming general NPDES permits. The DAR should seek a general NPDES permit for the use of rotenone and antimycin A for the control of invasive fish in certain Hawaiian freshwater habitats. Integrated fish control

protocols should be developed to guide managers through the decision making process and implementation of fish control projects.

In addition, the use of anesthetics as an adjuvant for mechanical removal of unwanted fish should be further considered. The application of MS-222, and AQUI-S (if and when this last product becomes approved for use in the US) could serve as an alternative to facilitate the capture of invasive tilapia in anchialine ponds for example. Experiments should be carried out to test this possibility and to verify potential side-effects, such as negative impacts to non-target invertebrates, environmental persistence and degradation rates.

The chemical control of established nuisance mollusk populations such as golden apple snails *Pomacea canaliculata* is still in its investigative phase. More research on alternative chemicals such as niclosamide and saponins needs to be carried out especially for the control of apple snails in taro patches. These investigations are expected to be carried out by academic researchers in Hawaii and elsewhere, and managers should keep themselves informed of new developments in this field through continued communication with representatives of groups of interest (e.g. Penny Levin; Dr. Robert Cowie).

Rapid response to eradicate early-detected introductions and to disinfect vessel hulls and other structures that could serve as vectors for new introductions can be accomplished by a combination of methods, including the use of chlorine and acetic acid. Other potential chemicals for these purposes include calcium hydroxide and hydrogen peroxide. These products have been used with success in the control of various biofouling taxa and, therefore, merit further exploration. Treatment side-effects can be minimized by combining the chemical method with neutralization products, containment technology and non-chemical treatment methods. There is an urgent need for further studies on the utilization of these products and auxiliary methods for rapid-response actions in Hawaii. The DAR should establish partnerships with research institutions (e.g. University of Hawaii, Aquenal Ltda.) and conduct experiments to generate the necessary information to establish rapid-response plans to combat potential next introductions. Specifically, research should address questions related to the efficacy, application rates and methods, degradation and dispersion rates of acetic acid, chlorine, calcium hydroxide and hydrogen peroxide when applied within containment units to combat some common AIS groups, such as tunicates, corallimorpharians, sponges, and mussels.

Under FIFRA, field experiments can be carried out without permits, if conducted on a total of not more than one surface-acre of water, provided: 1) when testing for multiple target species occurs at the same time and in the same locality, the one-acre surface limitation encompasses all target pest species; 2) the waters involved in or affected by the tests will not be used for irrigation, drinking water supplies or body-contact recreational activities; 3) the tests may not be conducted in waters which contain or affect any fish, shellfish, other animals, or plants taken for recreation or feed unless an appropriate tolerance or exemption from tolerance has been established. Acetic acid is registered by the USEPA as an herbicide but is not registered for aquatic applications. If experiments confirm that acetic acid is a valuable tool for rapid-response action, a Special Local Need registration (FIFRA Section 24c) may be sought from the USEPA through the DOA.

The major impediment for both experimental and rapid-response uses of acetic acid, as well as chlorine, calcium hydroxide and hydrogen peroxide are the CWA and state water quality requirements. Except for chlorine, for which state standard discharge limits exist, the application of all other potential chemical control methods to state waters is prohibited under HAR 11-54. A general NPDES permit and associated State Certification Waiver may be obtained from the USEPA/ DOH for experimental discharge of chlorine, but this process is expected to be burdensome. Current state regulation (HAR 11-54) establishes a numeric standard limit for chlorine discharges of 7.5-13 µg per liter of saltwater (>0.5 ppt) and 11-19 µg per liter of freshwater (<0.5 ppt), which restricts experiments with this active ingredient to applications done within containment units that allow for minimal leakage.

While many herbicides are registered for the control of invasive freshwater vegetation and algae, the options for the control of marine and brackish water species is limited to two active ingredients: glyphosate, for estuaries/ wetlands and imazapyr, for marine applications. These two systemic herbicides have been used to control invasive seagrasses and mangrove trees with relative success, but the control of invasive seaweeds continues unresolved. Chlorine (sodium hypochlorite) has been used in the eradication of a localized *Caulerpa taxifolia* invasion in California. A containment technology was used to guarantee sufficient contact of the chemical upon the target species and to minimize negative impacts on non-native species.

The list of successful eradication of AIS is short, especially in marine and brackish waters. However, the examination of such list can elucidate some important aspects of these efforts. *Terebrasabella heterouncinata* was eradicated from Cayucus, CA, using manual removal and containment filter system; monitoring program exists (Culver and Kuris 2000) (Juhasz, Moore et al. 2007). *Ascophyllum nodosum* was eradicated from SF Bay shoreline using manual removal (Miller, Andrew et al. 2004). *Undaria pinnatifida* eradicated from sunken vessel using heat plates and flame torch (Wotton, O'Brien et al. 2004). Chemical methods were successfully employed in rapid-response eradication of at least two species. *Mytilopsis* sp. was eradicated from three locked marinas in Darwin harbor using chlorine and copper sulphate. Fouled vessels were treated *in situ* where possible. Vessels exposed to the mussels but outside the marinas were surveyed and hauled onto the hard for at least a week if found to be carrying the mussel (Bax, Hayes et al. 2002). *Caulerpa taxifolia* eradicated from CA with sodium hypochlorite (Anderson 2005; Anderson, Tan et al. 2005).

A major common characteristic of these successful eradication projects is immediate management intervention. To make rapid-response possible a number of measures must be in place. In the paper that describes *C. taxifolia* eradication, Anderson (2005) remarks that the success of this project was due to (1) timely identification and notification of the infestation; (2) the proactive staff of the San Diego Regional Water Quality Control Board who deemed this invasion tantamount to an oil spill', thus freeing up emergency funding; (3) the mobilization of diver crews already working at the site. He also highlighted the fact that three well-integrated components of this rapid response have resulted in an effective eradication program: (a) expertise and knowledge on the biology of *C. taxifolia*; (b) knowledge on the uses, 'ownership' and characteristics of the infested site; (c) knowledge and experience in the implementation of aquatic plant eradication.

Dr. Anderson's remarks lead us to question our preparedness to deal with similar situations. Overall, I identify the urgent need for the establishment of decision frameworks for AIS control and eradication in Hawaii. These frameworks should include a pre-determined chain-of-command and list of authorities, responsibilities and permits associated to each group of potential new invasions and rapid-response type. Also, there should be a determination of specific source of funds to complete the rapid-response plan.

The framework should be comprised of a series of decision trees that could facilitate the many decisions that must be taken when dealing with rapid response, often in face of incomplete information.

Containment technologies must be designed, adapted and tested in combination with different chemical and non-chemical methods for the disinfection of vessels' hulls and also for on spot treatment of early detected introductions. Finally, risk assessments should be developed in order to forecast possible next invaders, and based on their biology, forecast the most suitable eradication and control options.

Next, I present my specific recommendations for the next actions I believe must be taken in order to give continuation to this project and make the chemical tools identified in this report available for AIS management in Hawaii:

Experiments

Laboratory/ microcosms experiments should be conducted to test the acute toxicity of acetic acid, chlorine, hydrated lime (calcium hydroxide), imazapyr, menadione (SeaKleen) and peracetic acid (Peraclean) to target and non-target species of interest (e.g. invasive seaweed species, tunicates and other biofouling species, invasive corals, corallimorpharians, invasive and non-target fish). The results from these tests should be used to determine which active ingredients can be applied to kill or inactivate newly detected introductions during rapid-response actions. I would recommend the DAR to establish collaboration/ partnership with UH researchers and/ or researchers from other institutions and localities (e.g. Aquenal Ltd., Australia) for two reasons: a) to have tests results validated by others and b) to be able to test species that are not found in Hawaii and that be representative of possible next invaders.

Dye experiments should be conducted with containment units available (i.e. IMProtector) to test dispersion and leakage rates. In addition, the DAR should invest in developing and testing new containment technologies. I would suggest DAR personnel to construct containment units based on the model utilized in California for the *Caulerpa taxifolia* rapid response action (see Chemical Toolbox for a description) and test these units with dyes.

Legal and Regulatory Matters

DAR personnel should decide on the terms of what the Division would like to have approved from the DOH in the General NPDES permits for aquatic pesticide applications that will be issued in April 2011. According to the timeline set by the USEPA for NPDES administrators within states (i.e. DOH in Hawaii), discussions with states and stakeholders on the General NPDES permits for aquatic pesticide were initiated in Sept./Oct. 2009 and proposed draft permits are due to be finalized in Apr./2010.

I suggest DAR to request a General NPDES permit that authorizes DAR personnel to use the various USEPA-registered aquatic pesticides for AIS control and eradication. Specifically, DAR's general NPDES permit should authorize DAR personnel to apply: rotenone and antimycin A for invasive fish control projects in freshwater environments, including ponds, streams and anchialine pools; imazapyr for invasive vegetation in shoreline area; all other USEPA-registered freshwater herbicides (See Chemical Toolbox for a complete list) for the control of the different types of freshwater invasive vegetation outbreaks (e.g. *Salvinia molesta*) in lakes, ponds, waterways. During these months that proceed the finalization of the General NPDES permits, the DOH will have to change two of their HARs, i.e. HAR 11-54 and HAR 11-55. DAR should try to follow this process to make sure these changes take place and that they are in line with the actions authorized by the General NPDES permits. This is very important, especially in light of what has happened the last time DOH made changes in their HARs (i.e. in 2004/05, the DOH changed their HAR 11-55 to authorize licensed applicators to use USEPA-registered aquatic pesticides without the need for an NPDES permit. This was done in order to make state regulations in harmony with the USEPA interpretation of the CWA. However, the DOH failed to change their HAR 11-54 and therefore, the changes made in HAR 11-55 were in practice, nullified).

DAR should initiate the development of a rapid-response protocol for the combat of newly detected AIS in freshwater and marine environments. The protocol should be developed in collaboration with the several other institutions that deal with invasive

species in the state; this includes USFWS, US Coast Guard, and DOA. The collaborative process to develop the AIS rapid-response protocol should be discussed within one or more of the formal existing councils and coordination bodies (i.e. CGAPS and HISC).

Once the protocol is designed, a Variance from DOH must be pursued. The Variance should authorize DAR personnel to use certain chemicals that are not registered by the USEPA for AIS control (e.g. acetic acid, chlorine) but that been given a SLN registration, in the case of a new AIS detection. The procedure and conditions for a Variance application are established in HRS §342D-7 (addendum 17). A copy of a Variance granted by the DOH to the US COAST GUARD AND OCEANIA REGIONAL RESPONSE TEAM for the Discharges of Oil Dispersants in case of Oil Spills (addendums 18-19) is available and should be used as a starting point for the Variance request for the discharge of chemical products for the control of newly detected AIS.

Also, the permits and special registration obtained by the Southern California *Caulerpa* action team (SCCAT; www.sccat.net) at the time of the *Caulerpa taxifolia* rapid-response should be analyzed and perhaps used as a model for the terms of the application for the AIS rapid-response Variance.

Appendix 1: Overview of Non-Chemical AIS Control Methods

Ideally, AIS control or eradication plan should be designed in an integrated manner, so that the utilization of chemicals can be minimized and overall program success is maximized and sustainable. Next, I review some common non-chemical control methods that can be considered as alternatives or adjuvants to chemical methods.

Dewatering

Aside from the use of pesticides, complete dewatering is the only method that can cause complete eradication of invasive animals (Lennon, Hunn et al. 1971; Finlayson, Schnick et al. 2000). The technique may also work for the control of aquatic vegetation, depending on the species and its tolerance to desiccation and air exposure.

Various types of pumps can be used for dewatering projects. For instance, trash pumps are usually the most suitable pump design because of the presence of solids in the water. Generally, trash pumps can handle spherical solids up to $\frac{1}{2}$ the diameter of the suction inlet. Trash pumps may be powered by diesel motors or electric motors (the last would require a coupled generator). Success of this method depends on the pumping capacity of the dewatering equipment used and also on the site's sediment permeability, which if very positive would counteract dewatering efforts and require extra pumping capacity. This gradient potential must be considered when calculating minimum pumping capacity of equipment and operation time. The best time of the year to carry out dewatering operations would be in the summer, when water levels are at their lowest.

The cost of trash pumps varies greatly (hundreds to tens of thousands of dollars) depending on pumping capacity, head, lift and maximum solid diameter the model can handle. Depending on specifications, pumps may be rented instead of purchased.

Some mechanical/physical AIS rapid response and control methods, such as dewatering might require Federal or state Section 404 permits. CWA Section 404 establishes a program to regulate the discharge of dredged and filling material into waters of the United States. Responsibility for administering and enforcing Section 404 is shared by the U.S. Army Corps of Engineers (USACE) and USEPA. USACE administers the day-to-day program, including individual permit decisions and jurisdictional determinations; develops policy and guidance; and enforces Section 404 provisions. EPA develops and interprets environmental criteria used in evaluating permit applications, identifies activities that are exempt from permitting, reviews/comments on individual permit applications, enforces Section 404 provisions, and has authority to veto USACE permit decisions.

USEPA and USACE have issued a rule stating that they regard the use of mechanized earth-moving equipment to conduct activities in waters of the United States (e.g. land clearing, ditching, channelization, and in-stream mining) as regulated discharge of dredged or fill material under Section 404 unless project-specific evidence shows otherwise. USACE regulatory program management and administration is focused at the District office level, with policy oversight at higher levels. District Engineers are authorized to issue permits, including standard permits, letters of permission, and regional general permits. Division Engineers may also issue permits under certain circumstances. USACE also issues nationwide permits that authorize certain activities that result in minimal adverse environmental effects. Natural resource managers should consult the appropriate USACE District office when planning AIS rapid response or control actions to determine if these actions require a Federal Section 404 permit.

Standard permits can be issued in situations where, after a public notice and comment period, the USACE District Engineer determines that the proposed activity is not contrary to the public interest. USACE issues a public notice within 15 days of receiving a completed permit application. The public notice describes the proposed activity, its location, and potential environmental impacts and invites comments within a specified time period, typically 15 to 30 days. The public at large, as well as interested Federal, state, and local agencies, have an opportunity to comment on the proposed activity.

Letters of permission can be issued in situations where the USACE District Engineer determines the proposed work would be minor, would not have significant

individual or cumulative impact on environmental values, and will not encounter appreciable opposition. Concerned fish and wildlife agencies and, typically, adjacent property owners who might be affected by the proposal are notified, but the public at large is not. Section 404 letters of permission can be issued only in cases where, after consulting with certain Federal and state agencies, the USACE District Engineer has previously approved categories of activities that can be authorized by letter of permission procedures.

Requesting a letter of permission may be an appropriate and relatively expedient means of complying with Section 404 for many relatively localized and non-controversial AIS rapid response or control actions that require Section 404 compliance.

General permits are often issued by USACE for categories of activities that are similar in nature and would have only minimal individual or cumulative adverse environmental effects. General permits can be issued on a nationwide (“nationwide permit”) or regional (“regional general permit”) basis. A general permit can also be issued on a programmatic basis (“programmatic general permit”) to avoid duplication of permits for state, local or other Federal agency programs. The mechanized clearing of riparian areas for the control of invasive species may be authorized by a nationwide permit, but the appropriate USACE District office should be contacted to determine if a nationwide permit can be used to authorize a specific activity. In some USACE Districts, nationwide permits have been suspended or revoked, and Section 404 standard permits, letters of permission, regional general permits, or programmatic general permits are used instead. In general, to obtain a Section 404 permit, applicants must demonstrate that the discharge of dredged or fill material would not significantly degrade the nation’s waters and there are no practicable alternatives less damaging to the aquatic environment. Applicants should also describe steps taken to minimize impacts to water bodies and wetlands and provide appropriate and practicable mitigation, such as restoring or creating wetlands, for any remaining, unavoidable impacts.

Permits will not be granted for proposals that are found to be contrary to the public interest. In the case of AIS rapid response or control actions, the removal of AIS or mitigation of their harmful effects could be considered a benefit of the action. Compliance with the Endangered Species Act and/or Section 106 of the National Historic Preservation Act may also be required before a Section 404 permit can be issued.

On average, individual permit decisions (standard permits and letters of permission) are made within 2 to 6 months from receipt of a completed application. For activities authorized by general permits, decisions are usually made in less than 30 days. In emergencies, USACE may be able to expedite the permitting process. Natural resource managers considering AIS rapid response actions should contact their District Engineer to discuss the circumstances and request use of expedited procedures. Expedited procedures are authorized on a case-by-case basis. Permit applications that require the preparation of an Environmental Impact Statement take an average of 3 years to process.

Manual and mechanical removal

As presented here, manual and mechanical methods of removal, includes direct and indirect collection of AIS from intertidal or submerged substrata. These types of removal may be carried out with the use of divers, volunteers or other personnel and may utilize subaquatic equipment to pressure wash, dig, drag, suck up, scrape or pump AIS out of the water.

Manual removal of AIS is probably the most common form of AIS control effort around the world. It is usually associated with low treatment risks and few side-effects, but also with low treatment efficiency. Nevertheless, successful cases of AIS manual and mechanical removal exist, especially when the intervention happens immediately upon discovery of a new introduction. The introduced sabellid polychaete *Terebrasabella heterouncinata* was eradicated from Cayucus, CA, using manual removal and containment filter systems (Culver and Kuris 2000; Juhasz, Moore et al. 2007). The brown alga *Ascophyllum nodosum* was also eradicated from the San Francisco Bay shoreline also using manual removal (Miller, Andrew et al. 2004).

Examples of manual and mechanical removal of AIS in Hawaii include volunteer programs to remove invasive weeds from intertidal and shallow waters. Algae Clean-up events, such as the one that took place on 24 August 2002 in front of Waikīkī Aquarium, organizes volunteers to remove massive amounts of unwanted seaweed from the water. In this specific event, five thousand pounds of *Gracilaria salicornia* algae were

removed on one day and as of late 2005, the 'A'ohē Limu'e, No Alien Algae program had removed about 100 tons of *Gracilaria* from the area (see http://www.hawaii.edu/malamalama/2006/01/f4_algae.html [accessed February 2007]).

Another example of AIS mechanical removal is the removal of invasive algae with the use of the Supersucker. The Supersucker is a mechanical suction device for invasive algae control that has been developed by a collaboration of organizations interested in invasive algae management control. This collaboration (called HIMAG, or the Hawaii Marine Algae Group) includes Hawaii Department of Land & Natural Resources/Division of Aquatic Resources, The Nature Conservancy and the University of Hawaii. The Supersucker has been deployed in Kaneohe Bay, on the windward side of Oahu, and consists of a pump on a barge that is used to gently pull algae from the reef surface, where it is deposited on a screened sorting table. The crew of 5-8 divers and operators are capable of removing up to 750 lbs. of alien algae per hour (or up to 3000 lbs. per day). A smaller, mobile version (dubbed "Supersucker Jr.") has been constructed and has been used at various locations around Oahu.

Some mechanical/physical AIS rapid response and control methods, such as those described above might require Federal or state Section 404 permits (see more information in the Dewatering Section above).

Electrofishing

Electrofishing consists of using electric fields in water to stun fish and facilitate their capture. It is commonly used as a surveying method and does not normally kill fish, but mortality may occur depending on type, intensity, duration, orientation of current and species physiology. There are three basic types of electrofishing equipment: backpack, towed barge, and boat mounted electrofisher. Electrofishing efficiency is influenced by various factors the most important one being water conductivity which is directly related to salinity. With everything staying equal, more power is required to achieve effective voltage in saltwater than in freshwater which limits the methods' applicability in marine (30-50 ppt) and brackish (1-30 ppt) environments. High water temperature and small fish size (body length) also limit electrofishing efficiency. If electrofishing is not supervised by DAR/DLNR staff, a

Special Activity Permit issued by the DAR/ DLNR is required in accordance with HRS §187A-6, Chapter 195D. A Special Activity Permit may take up to 45 days to process.

Currently, the DAR owns 1 boat-mounted electrofishing unit that is kept at Sandy Island, Oahu, and a number of backpack units. Safety gear for personnel should include rubber boots and appropriate clothing. Batteries and generators, cables and connectors may be required. Equipment such as buckets, landing net handles and fish containers, must be made of non-conducting material as far as is reasonably practicable.

Electrofishing requires at least two people: one to operate the anodes, and another to catch the stunned fish with a dip net. If using a boat, at least one extra person is required to drive it. More help may be needed to collect and transport fish during electrofishing operation. Personnel needs would vary with the number and area of ponds that qualify for this treatment.

Blasting

Detonation cord is a flexible, rope-like material containing an explosive core, usually pentaerythritol tetranitrate (PETN). It has a variety of applications in the mining and construction industries. Most applications of explosives in fisheries have been carried out for sampling purposes (Metzger and Shafland 1986; Bayley and Austen 1988; Keevin, Hempen et al. 1995), but at least two experiments looked at the efficacy of explosives to eradicate invasive fish: Johnston (1961) used dynamite to kill longnose gar (*Lepisosteus osseus*) from large coastal streams in North Carolina, and the California Dept. of Fish and Game used detonation cords to kill Northern Pike in Lake Davis, California (California Department of Fish and Game 2002). Efficiency of blasting as a method to kill fish depends on the depth and type of bottom sediment of the site (Bayley and Austen 1988; Paulsen 2002).

Federal regulatory guidelines for the handling and detonation of explosives are set by the Mining Enforcement and Safety Administration (MESA), the Federal Bureau of Alcohol Tobacco, and Firearms (ATF) and the Mining Safety and Health Administration (MSHA). Local regulations for hazardous materials and explosives are set by the Hawaii Occupational Safety and Health Division (HIOSH). At least the detonation team leader must

have a Certificate of Fitness for Explosives in accordance with HAR Section 12-58-1. In addition, if blasting is not supervised by DAR staff, a Special Activity Permit (HRS§187A-6) must be obtained from DAR.

Primacord® is usually measured in grams of PETN per meter of detonating cord, or in number of grains of explosive per foot. One grain of explosive contains approximately 0.06 grams of PETN (Dyno Nobel Inc. 2009). Based on the experiments mentioned above (California Department of Fish and Game 2002), we estimate that treating a 2 acre-pond would require approximately 2,500 ft. of 50 grain per ft. of Primacord. To calculate this figure we assumed that the cords are laid in a 400 ft x 40 ft. rectangle in deeper areas and that explosion kills fish at a maximum distance of ~ 30 ft from the cord. Primacord® comes wrapped on 1,000-foot spools. Other materials needed: steel posts to set and suspend cords, electric blasting caps to ignite the cord; submerged “cars” containing sentinel fish for control purposes. At least one licensed and experienced detonation agent is required. Divers are usually needed to attach cords to poles and this way keep explosives suspended underwater.

Biocontrol

Biocontrol is the introduction or enhancement of predators, competitors, parasites or pathogens of the target invasive species. In general, biocontrol practices present low risks to human health, can be inexpensive and tend to be well accepted by the general public. However, examples of biocontrol programs that have backfired causing long-term negative environmental impacts abound (Ricciardi 1998; Bax, Carlton et al. 2001). Post-introduction changes in behavior and physiology of biocontrol agents can cause adverse outcomes when either exotic or native species are used.

Mass stocking of a predator species may reduce populations of invasive animals and vegetation but is unlikely to result in complete eradication.

One example of a biocontrol effort that is currently being investigated in Hawaii is the enhancement of sea urchin populations in areas infested by invasive seaweeds. The objective of this program is to introduce sea urchins on reef areas that have

been cleared with the Supersucker in order to reduce the amount of fragments left behind by the mechanical removal. Seaweed fragments, especially in the case of the invasive algae *Kappaphycus* spp. propitiate rapid recolonization, defeating the mechanical removal effort.

If utilizing native or domesticated species, no specific permit is required. However, if transporting fish species from one island to another, an authorization may be necessary from Department of Agriculture. If introduction of non-native species is not supervised by DAR staff, a Special Activity Permit (HRS§ 187A-6) must be obtained from the DAR/ DLNR.

High costs would probably be associated with preliminary tests and experiments. Bioassays (in lab and *in situ*) should be performed to test potential biocontrol agents' effectiveness, determine number of individuals that should be released, and identify risks.

Temperature manipulation

The spraying of hot or cold water onto patches or colonies of AIS is also an alternative that merits consideration. Ambient temperature manipulation has been used with success against other aquatic invasive species. For instance, hot water was tested as a treatment method to eliminate gametophyte banks of the seaweed *Undaria* sp. in New Zealand, and was found to be very effective at temperatures of 60°C and 95°C. Gametophyte exposure times of 5 seconds, 10 seconds and 60 seconds produced 100 % mortality. On the basis of these results a diver hand held hot water sterilization system was developed. Hot water was heated by a diesel burner up to a maximum temperature of 150 °C using fresh water pumped from shore and/or stored in a tank on the support vessel. It was delivered to a diver operated lance via an insulated hose. Low pressure was used to minimize the amount of material dislodged during the sterilization process and avoid the possibility of dispersing *Undaria* gametophytes. The lance was fitted with a funnel to help concentrate the hot water, and temperatures were monitored by a temperature probe inside the funnel that was connected to a data logger for later analysis. Prior to the sterilization of an area, rock and wooden surfaces infested with *Undaria* gametophytes were stripped of all macroinvertebrates and macroalgae. Hot water was used to treat shoreline infested areas of

Halfmoon Bay and in Big Glory Bay at the Nugget. This technique was also modified and used successfully to treat and eradicate *Undaria* from a sunken fishing vessel (Seafresh I) at the Chatham Islands (Hunt, Chadderton et al. 2009).

While the release of hot water associated with AIS control activities such as the one described above into the ambient water is likely to cause minimal or no significant harm to non-target species, this release constitutes a discharge which may trigger some laws and regulations that deal with water quality control, specifically the CWA (US Code Titel 33 Chap. 26), the State Water Pollution Control Law (HRS 342D) and its associated rules (HAR 11-54 and 11-55). Both the CWA and HRS 342D define pollution as any “man-made or man-induced alteration of the chemical, physical, biological, and radiological integrity of water”, which includes hot water. HAR 11-54-4(a)(4) states that “(a) All waters shall be free of substances attributable to domestic, industrial, or other controllable sources of pollutants, including (...) (4) High or low temperatures (...) at levels or in combinations sufficient to be toxic or harmful to human, animal, plant, or aquatic life, or in amounts sufficient to interfere with any beneficial use of the water and HAR §11-54-9 determines that the temperature in all waters of the state (except for Zones of mixing, also defined in this HAR) shall not vary more than one degree Celsius. Therefore, in order to utilize water temperature manipulation, one must seek an NPDES permit and associated Water Quality Certification from DOH. The need for an NPDES permit and compliance with water quality standards can only be waived through a Variance (HRS 342D-7) or if a nationwide or regional NPDES permit authorization exists for this type of discharge (HAR 11-54-9.1.04).

Salinity manipulation

The use of high and low salinity waters to kill or inactivate unwanted aquatic species relies on the concept of species tolerance to changes in the amount of dissolved salts in ambient water and species’ limited capacity to adapt their osmoregulation process to these changes. For instance, the major ballast water treatment currently implement worldwide, ballast water exchange, is based on this concept of organisms’ salinity tolerance. It consists of requiring transoceanic vessels to exchange their ballast water, which usually contains river and estuarine organisms adapted to low salinity conditions, for mid-ocean

water. The logic behind this practice is that estuarine and freshwater organisms dumped out in mid-ocean will die upon contact with the high salinity of open ocean water, while the oceanic water collected will have organisms that will also die when dumped at the port of arrival which is expected to have ambient waters of lower salinity (Sutherland, Levings et al. 2001). Organisms' salinity tolerance, however, is highly variable amongst taxa and populations. For instance, saline tolerance range is moderate for most reef organisms, including hermatypic scleractinian corals, which live with continuous salinity exposure of 25-42 ppt, but is expected to vary among species (Coles and Jokiel 1992).

Legal and regulatory requirements for this practice are expected to be similar to those for temperature manipulation (above).

Nets and traps

In some circumstances it is possible to adapt fishing gear to capture invasive fish and mollusks, although more often than not, invasive fish species are hard to catch with nets and traps. A number of nets and traps exist and the choice should be made to match the size, shape and behavior of the target species.

For instance, tilapias are adept at escaping seine nets by jumping over or burrowing under it. According to experts in aquaculture, tilapias are best harvested by seining and draining the pond and complete harvest is not possible by seining alone. Only 25- 40% of a *T. nilotica* population could be captured per seine haul in small ponds. Other tilapia species, such as *T. aurea*, are even more difficult to capture (Rakocy and McGinty 2005). Alternatively, an adaptation of a beam bottom trawling net could be experimentally used to capture tilapia. A beam bottom trawling net is a net in which the mouth is held open by a solid metal beam, attached to two solid metal plates welded to the ends of the beam, which slide over and scrape the bottom sediment as the net is pulled manually or by a small boat.

Minnow traps are cylindrical, double-ended wire or plastic mesh funnels that narrow in the middle. They work on the assumption that fish will swim into the trap to eat baits and will not be able to find the way out. However, various fish species have been

observed entering and exiting traps (Kocot, Baldwin et al. 2005) and efficiency of minnow traps varies greatly with environmental and biological factors (Blaustein 1989). In addition, the cost of equipment and man power that would be required to install, maintain, remove and replace sufficient numbers of traps to remove the large number of tilapia established in the site is expected to be prohibitive.

If small mesh size used for this purposes (<2 ¼ inch of stretched mesh), a Special Activity Permit must be issued by DLNR/ DAR (HAR §13-75), unless operation is supervised by DAR staff.

Smothering

Smothering control techniques consist of utilizing wrapping and other containment units in order to restrict the target species' access to oxygen, food particles and light in the case of photosynthesizing organisms.

Since 2001, the DAR has been engaged in exploring possible ways to control the spread of *Carijoa riisei* in the Hawaiian archipelago. In an attempt to destroy *C. riisei* in Port Allen Harbor, Kauai, 738 pier pilings were wrapped with special plastic tarps (i.e. 4 mm black poly sheeting and 90 gauge Econowrapper stretchwrap plastic). This smothering technique had shown success in the eradication of the colonial tunicate *Didemnum vexillum* in New Zealand (Coutts and Forrest 2007) and seemed promising against *C. riisei*. So far, this method does not seem to be an efficient way to remove the target populations. Nevertheless, the utilization of this smothering technique could be revisited, if more suitable wrapping and enclosing material and technique could be identified, and perhaps combined with other control methods such as hot/ cold water or freshwater treatments.

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Glossary

Absorption: The uptake of water or dissolved chemicals by a cell or an organism; the movement of a chemical into or across a tissue; the uptake of chemicals by a cell or an organism; the movement of a chemical into or across a tissue.

Acceptable Daily Intake (ADI): The maximum dose of a substance that is anticipated to be without lifetime risk to humans when taken daily.

Accumulation: bioaccumulation; the build-up of a chemical in the body due to a long-term or repeated exposure; the buildup of a chemical in the body due to long-term or repeated exposure.

Acetylcholinesterase: An enzyme present in nerve tissue, muscles and red blood cells that catalyzes the hydrolysis of acetylcholine to chlorine and acetic acid; true cholinesterase.

ACGIH: American Conference of Governmental Industrial Hygienists, which establishes exposure limits for workers.

Acid equivalent (a.e.): The amount of active ingredient expressed in terms of the parent acid.

Activation: In mutagenicity testing, the exposure of the tested chemical to a source of enzymes, e.g., liver microsomes, which metabolize the chemical. The metabolic products may exhibit mutagenic activity where the original chemical did not.

Active ingredient (a.i.): a chemical compound in a pesticide formulation that produces the pesticide effect in the target species; the agent primarily responsible for the intended pesticidal effects of a product.

Active transport: An energy-expending mechanism by which a cell moves a chemical across the cell membrane from a point of lower concentration to a point of higher concentration, against the diffusion factor.

Acute: One exposure or short-term exposure; used to describe brief exposures and effects which appear promptly after exposure.

Additive Effect: Combined effect of two or more chemicals equal to the sum of their individual effects (on the same function or organism).

Adjuvant: Substance added to a spray to act as a wetting or spreading agent, sticker, penetrant, or emulsifier in order to enhance the physical characteristics of the pesticidal materials.

Adsorption: adhesion of the molecules of gases, liquids, or dissolved substances to the surface of, or into pores of a solid surface, without chemical reaction; the process by which chemicals are held on a solid surface.

Aerobic: A process requiring oxygen or free air.

Algistatic: that prevents new algal growth

Algaecidal: that kills existing algae

Ambient: Environmental or surrounding conditions.

Anaerobic: a process that does not require oxygen or a condition in which oxygen is absent.

Antagonism: The combined action of two or more substances to produce an effect less than the sum of their individual effects; the opposite of synergism.

Assay: A test for a particular chemical or effect.

Atrophy: The wasting away or reduction in the size of a cell, tissue, or organ(s).

Background level: Normal environmental concentration of a chemical.

Basic: alkaline; having a pH greater than 7

Bias: An inadequacy in experimental design that leads to results or conclusions not representative of the population under study.

Bioaccumulation: The absorption and concentration of a substance in plants or animals upon environmental exposure.

Bioassay: Test which determines the effect of a chemical on a population of living organisms.

Biocide: a material that has the capacity to kill forms of life.

- Bioconcentration:** The accumulation of a chemical in tissues of an organism (such as fish) to levels that are greater than the level in the medium (such as water) in which the organism resides.
- Bioconcentration Factor (BCF):** A compound is considered likely to bioconcentrate if the bioconcentration factor is >1000; a measure of the tendency for a chemical to accumulate
- Biodegradation:** Decomposition of a substance into more elementary compounds by the action of microorganisms such as bacteria.
- Biomagnification:** The serial accumulation of a chemical by organisms in the food chain, with higher concentrations of the substance in each succeeding trophic level. [Note (JWG): Pertains to only those trophic levels which demonstrate biomagnification, which does not necessarily occur in all situations.]
- Biopesticide:** a pesticide that is organic, as opposing to synthetic.
- Carcinogen:** Any substance capable of producing cancer or a chemical which causes or induces cancer.
- Carcinogenicity:** The ability of a substance to produce malignant tumors.
- Carrier:** Material added to an active ingredient to facilitate its preparation, storage, shipment, or use.
- CAS No.:** Chemical Abstracts Service Registry Number. The CAS No. is assigned to a specific compound and is used for cross-referencing the various chemical names which have been used for the same compound.
- Central nervous system:** Portion of the nervous system which consists of the brain and spinal cord; CNS.
- Cholinesterase:** An enzyme of the body necessary for proper nerve function; also called serum cholinesterase.
- Chromosomal aberration:** An irregularity in the number or constitution of chromosomes that may alter the development of the embryo.
- Chromosome:** Rod-like structure in the nucleus of a cell that forms during mitosis; composed of DNA and protein; chromosomes contain the genes responsible for heredity.
- Chronic:** Occurring over a long period of time, either continuously or intermittently, used to describe ongoing exposures and effects that develop only after a long exposure.
- Ciliary:** Related to the suspension of the lens of the eye. [Note(JWG): Also movement of projections from cells in the lung or certain microorganisms.]
- Clinical studies:** Studies of humans under special conditions.
- Common names:** Common names for pesticides are established or accepted by the following organizations: ANSI - American National Standards Institute; BSI - British Standards Institution; ISO - International Organization for Standardization; WSSA - Weed Science Society of America. A common name generally is not accepted for use on EPA registered labels or in CFR regulations until it has been accepted by ANSI.
- Concentration:** The amount of active ingredient or pesticide equivalent in a quantity of diluent, expresses as lb/gal, gL-1, mLL-1, etc.
- Concentration factors:** Concentration factors indicate whether a compound is accumulated in the tissue of an organism. It is calculated by dividing the concentration of the compound in the tissue by the concentration ingested in the diet or taken up from the surrounding medium.
- Confounding factors:** Variables other than that being tested which can affect the incidence or degree of a parameter being measured.
- Congenital:** Existing at birth but acquired in the uterus rather than inherited.
- Conjugate:** A compound resulting from the bonding of two other compounds. Examples include glucoside conjugates formed from pesticides in plants and glucuronide conjugates formed from pesticides in animals.
- Control group:** A group of experimental subjects which is not exposed to a chemical or treatment being investigated; compared to experimental group which is exposed to chemical or treatment.

Cost/benefit analysis: A quantitative evaluation of the costs which would be incurred versus the overall benefits to society of a proposed action such as the establishment of an acceptable dose of a toxic chemical.

Cumulative exposure: The summation of exposures of an organism to a chemical over a period of time.

Cytological aberration: A deviation from normal cell structure or function.

Cytotoxicity: The ability of an agent to interfere with cell metabolism.

Decomposition: or degradation; a chemical alteration of the pesticide, chemical or biological breakdown of a complex compound into simpler compounds.

Degradation: same as decomposition.

Demography: The study of the characteristics of human populations such as size, growth, density, distribution, and vital statistics.

Demyelination: The destruction or removal of the myelin sheath which is composed of a lipid substance and envelops certain nerve fibers.

Dermal: Of the skin; through or by the skin.

Diffusion: The movement of suspended or dissolved particles from a more concentrated to a less concentrated region as a result of the random movement of individual particles; the process tends to distribute them uniformly throughout the available volume.

Distribution: The movement of a chemical from the blood to other tissues.

Dominant lethal assay: A mutagenic bioassay used in assessing the ability of a chemical to penetrate gonadal tissue and produce genetic damage. Male animals are treated with a test substance acutely (single dose) or over the entire period of sperm production. These males then are mated with females, which after about the 14th day of pregnancy are sacrificed and examined for number of total implantations and viable fetuses.

Dose response: A quantitative relationship between the dose of a chemical and an effect caused by the chemical.

Dose: A measure of exposure. Dose is often expressed in milligrams per kilogram (mg/kg) or parts per million (ppm). [Note (JWG): Infers actual uptake.]

Ecology: The study of the interrelationships between living organisms and their environment, both physical and biological.

Ecosystem: The interacting system of a biological community and its nonliving environment.

Effective Dose: The ED₅₀ is the effective dose for 50 percent of tested subjects.

Elimination: The removal of a chemical from the body in urine, feces, or expired air.

Embryo: The developing animal during pregnancy.

Embryotoxicity: A compound-induced toxic effect on the embryo during the initial phase of pregnancy.

Endangerment assessment: A site-specific risk assessment of the actual or potential danger to human health or welfare and the environment from the release of hazardous substances or waste.

Endpoint: A biological effect used as an index of the effect of a chemical on an organism.

Environmental fate: The destiny of a chemical after release to the environment; involves considerations such as transport through air, soil and water, bioconcentration, degradation, etc.

Enzyme: A protein, synthesized by a cell, that acts as a catalyst in a specific chemical reaction.

Epidemiological: Having to do with the study of the incidence and distribution of disease or toxicity.

Eudicotyledon: an angiosperm having two cotyledons in the seed, leaves with a network of veins radiating from a central main vein, flower parts in multiples of four or five, and a ring of vascular cambium in the stem; undergo secondary growth.

Excretion: The removal of a chemical from the body in urine, feces, or expired air.

Exposure: Contact with a chemical. Some common routes of exposure are dermal (skin), oral (by mouth), and inhalation (breathing).

Extrapolation: Estimation of unknown values by extending or projecting from known values.

FDA Monitoring: The collection and analysis for pesticide residues carried out by the Food and Drug Administration. The primary components of the monitoring are the Total Diet Studies, which entail examining ready-to-eat foods, and the Compliance Programs, special assignments, and surveys on both domestic and imported foods, which include both surveillance and compliance examinations of fresh fruits and vegetables, grains, milk and dairy products, fish, and a variety of processed products and by-products.

Fetal resorption: Following the in utero death of a fetus, for whatever reason, the fetal tissues will undergo either partial or complete dissolution and the resulting products will be absorbed by the maternal tissue.

Fetotoxicity: A compound-induced toxic effect on the fetus during the latter phase of pregnancy.

Gametophyte: Multicellular structure, or phase in plants and algae that undergo alternation of generations, that is haploid.

Gastrointestinal tract (GI Tract): The entire digestive canal from mouth to anus.

Genotoxicity: Any toxic modification or alteration of the structure or function of genetic material.

Gestation: The duration of pregnancy. In the human, gestation is normally nine months.

GRGL: Groundwater residue guidance level.

HA: Health advisory.

Half-life: The time required for half of the residue to lose its analytical identity whether through dissipation, decomposition, metabolic alteration or other factors. The half-life concept may be applied to residues in crops, soil, water, animals or specific tissues.

Haploid: That contains only one complete set of chromosomes.

Hazard: The potential that the use of a product would result in an adverse effect on man or the environment in a given situation.

H_c (atm-cubic meter/mole) = Vapor Pressure (atm) x Mole Wt (g/mole) /Water Solubility (g/m³)

Hematopoiesis: The production of blood and blood cells; hemopoiesis.

Hemotoxicity: A toxic effect on blood components or properties such as changes in hemoglobin, pH, electrolytes, or protein of the plasma.

Henry's Law Constant: A parameter used in evaluating air exposure pathways. Values for Henry's Law Constant (H_c) were calculated using the following equation and the values previously recorded for solubility, vapor pressure, and molecular weight:

Hepatoma: A malignant tumor occurring in the liver.

Herbicide: A chemical used to kill plants.

Histology: The study of the structure of cells and tissues; usually involves microscopic examination of tissue slices.

Homeostasis: Maintenance of a constant internal environment in an organism.

Hormone: A chemical substance secreted in one part of an organism and transported to another part of that organism where it has a specific effect.

Host-mediated assay: This assay evaluates the genotoxicity of a substance to microbial cells introduced (e.g., by intravenous injection) into a host animal. The host animal receives the test compound orally, and therefore acts as a source of chemical metabolism, distribution and excretion.

Human equivalent dose: A dose which, when administered to humans, produces an effect equal to that produced by a dose in animals.

Hydrology: The study of the properties, distribution, behavior and effects of water on the earth's surface, in the soil and underlying rocks and in the atmosphere.

Hydrolyze: The chemical process of the pesticide breaking down or decomposing by the splitting of the molecule and addition of a water molecule in a process of hydrolysis.

Hypoesthesia: Decreased sense of touch.

Hypoxia: A deficiency of oxygen.

IC₅₀: Median inhibitory concentration or dose; a measure of the effectiveness of a compound in inhibiting biological or biochemical function in half of the test organisms.

Immunomodulation: Change in the body's immune system, caused by agents that activate or suppress its function.

In situ studies: Studies performed in the natural ambient of the test subject; not in a laboratory or experimental facility.

In vitro studies: Studies of chemical effects conducted in tissues, cells or subcellular extracts from an organism (i.e., not in the living organism).

In vivo studies: Studies of chemical effects conducted in intact, living organisms.

Inhalation: Drawing of air into the lungs.

Insecticide: A chemical used to kill insects. [JWG: A chemical used to control pest insect populations.]

Intake: Amount of material inhaled, ingested, or absorbed dermally during a specified period of time.

Intraperitoneal (I.P.): The introduction (e.g., by injection) of a substance into the peritoneal cavity which is comprised of the abdominal and pelvic spaces and contains the large internal organs.

Intubation: The insertion of a tube; for example, the passing of a tube from the mouth through the esophagus and into the stomach as a means of facilitating the accurate, oral dosing of a test animal with a substance.

Irreversible: Permanent, incurable.

L₅₀: lethal concentration or dose required to kill half the test organisms.

Larvicide: Usually refers to chemicals used for controlling mosquito larvae, but also to chemicals for controlling caterpillars on crops.

Latency: Time from the first exposure to a chemical until the appearance of a toxic effect.

LC: Lethal concentration.

LC50: The concentration of toxicant necessary to kill 50 percent of the organisms being tested. It is usually expressed in parts per million (ppm); The size of a single dose of a chemical necessary to kill 50 percent of the organisms in a specific test situation. It is usually expressed in the weight of the chemical per unit of body weight (mg/kg). It may be fed (oral LD50), applied to the skin (dermal LD50), or administered in the form of vapors (inhalation LD50).

Leach: The movement of a pesticide chemical or another substance downward through soil as a result of water movement, potentially causing contamination of groundwater resources.

LEL: Lowest effect level. In a series of dose levels tested, it is the lowest level at which an effect is observed in the species tested.

Lethality: Death.

LOAEL: Lowest-Observed-Adverse-Effect-Level; the lowest dose in an experiment which produced an observable adverse effect.

LOEL: Lowest-observed-effect-level.

MATC: Maximum acceptable tolerance concentration. [Note (JWG): The geometric mean of the NOEL and the LOEL for chronic exposure, usually of aquatic organisms.]

MCL: Maximum contaminant level.

Metabolite: Any product of metabolism, especially a transformed chemical.

mg/kg/day: Milligrams per kilogram per day.

mg/kg: Milligrams per kilogram.

mg/L-1: Milligrams per liter

mg/m³: Milligrams of material per cubic meter of air.

Miosis: Decreased pupil size.

MLD: Minimum lethal dose; the smallest of several doses which kills one of a group of test animals.

mm Hg: Millimeters of mercury. [tor]

Modeling: Use of mathematical equations to simulate and predict real events and processes.

Monitoring: Measuring concentrations of substances in environmental media or in human or other biological tissues.

MOS: Margin of safety.

- MTD: Maximum Tolerated Dose; the highest dose of a chemical that does not alter the lifespan or severely affect the health of an animal.
- Mucous Membranes: Any tissue lining body cavities and canals which come in contact with the air, and kept moist by secretions of various types of glands (e.g., in the mouth).
- Mutagen: An agent that causes a permanent genetic change in a cell other than that which occurs during normal genetic recombination.
- Mutagenicity: The ability of a substance to produce a detectable and heritable change in genetic material which may be transmitted to the progeny of affected individuals through germ cells (germinal mutation) or from one cell generation to another within the individual (somatic mutation).
- Mutation: An alteration in genetic structure which is passed from one generation to the next.
- Mydriasis: An excessive dilation of the pupil of the eye.
- Necropsy: The examination of a dead body, autopsy. The term necropsy is often used with respect to examination of animals other than humans.
- Necrosis: Death of cells or tissue.
- NEL: The no-effect-level of a pollutant is the concentration at or below which there will be no defined effect, either deleterious or beneficial, on a member of a population exposed to the pollutant in question.
- Neurotoxicity: The ability of a substance to destroy nerve tissues or affect behavior.
- NIOSH: National Institute of Occupational Safety and Health; a branch of the U.S. Department of Health and Human Services and the U.S. Department of Labor. Responsible for setting limits for exposure which workers should be allowed to receive in their work.
- NOAEL: No-Observed-Adverse-Effect Level; the highest dose in an experiment which did not produce an observable adverse effect.
- NOEL: The dosage or exposure level at which no toxicologically significant adverse effect(s) can be detected. The NOEL has been used interchangeably with the NEL (no effect level). However, there is a distinction which is based on the interpretation of the occurrence of an effect. An NEL denotes that at a particular dose, there was absolutely no effect. In reality, an effect may have occurred but went undetected for a variety of reasons. Thus, the more accepted terminology is the NOEL, which indicates that while an effect was not observed under a particular set of test conditions, it does not preclude the possibility that some effect may have occurred.
- Nontarget organisms: Any plant, animal, or other live organism which is not intended to be treated with a pesticide application; beings that, because they are either beneficial or harmless, are not to be killed by the pesticide.
- Non-volatile: Will not vaporize or become a gas.
- Oncogenicity: the ability of a substance to produce either benign or malignant tumors.
- Oral: Of the mouth; through or by the mouth.
- Organic: relating or belonging to the class of chemical compounds having a carbon basis; "hydrocarbons are organic compounds".
- Organogenesis: The time period during embryonic development during which all major organs and organ systems are formed. During this period, the embryo is most susceptible to factors interfering with development.
- Pathogen: Any disease-causing agent, usually applied to living agents; causing or capable of disease.
- Pesticide: A chemical used to kill pests. The two most common classes of pesticides are insecticides and herbicides.
- pH: The [negative logarithm of the] hydrogen ion concentration. [pH 7 is neutral, pH 1 is very acid, and pH 12 is very basic.]
- Phosphorylation: the addition of a phosphate (PO₄) group to a protein or other organic molecule; it activates or deactivates many protein enzymes, causing or preventing the mechanisms of diseases such as cancer and diabetes.

Phototoxicity: Toxicity resulting from sequential exposure to a photosensitizing agent and sunlight.

Physiological: Having to do with the mechanics of body function.

Potency: A measure of the relative strength of a chemical.

Potentiator: The ability of a substance to increase the toxic effect(s) of another compound.

ppb: Parts per billion; measure of concentration; [= ugL⁻¹ or ug/kg]; (1 ppb = 1 µgL⁻¹)

ppm: Parts per million; a measure of concentration; [= mgL⁻¹ or mg/kg].

Qualitative: Descriptive of [nature and relative] size, magnitude or degree.

Rate: The amount of active ingredient or acid equivalent applied per unit area or other treatment unit; received environmental exposure to a chemical.

Receptor: (1) In biochemistry: a specialized molecule in a cell that binds a specific chemical with high specificity and high affinity; (2) In exposure assessment: an organism that receives, may receive, or has

Renal: Pertaining to the kidney.

Reproductive Effects: Changes which may occur during the reproductive process including mutagenesis, teratogenesis, diminished fertility, death, growth retardation, functional disorders, and prematurity or death of the offspring.

Reservoir: A tissue in an organism or a place in the environment where a chemical accumulates, from which it may be released at a later time.

Residue: That quantity of pesticide, its degradation products, and/or its metabolites remaining on or in the soil, plant parts, animal tissues, whole organisms, and surfaces.

Risk assessment: A qualitative or quantitative evaluation of the environmental and/or health risk resulting from exposure to a chemical or physical agent (pollutant); combines exposure assessment results with toxicity assessment results to estimate risk.

Risk: The potential for realization of unwanted negative consequences or events.

RRfd: Risk reference dose.

Sensitization: The development of a hypersensitive or allergic reaction upon re-exposure to a substance. The reaction may be immediate or delayed and may be of acute, short term, or chronic duration.

SMAV: Species Mean Acute Value; the geometric mean of the results of all tests in which the concentrations of test material were measured.

SNARL: Suggested No Adverse Response Level.

Soil mobility: Movement of a compound through soil from the treated area by leaching, volatilization, adsorption and desorption, or dispersal by water. Leaching is of particular concern because of the potential for contamination of groundwater.

Solubility is a physical end point useful for understanding potential environmental impact. High water solubility is frequently associated with mobility and affects distribution in water and soil. This endpoint is determined for the active ingredient in a product and is typically reported as grams per 100 ml water at 25°C. The solubility of the active ingredient is minimal in water but is moderately soluble in alcohols, ether, chloroform and acetone.

Solvent solubility: Concentration that dissolves in a given solvent.

Sorption: A surface phenomenon which may be either absorption or adsorption, or a combination of the two; often used when the specific mechanism is not known.

Statistically significant: Probably caused by something other than mere chance.

STEL: Short-term exposure limit. The maximal allowable concentration, or ceiling, not to be exceeded at any time during a 15-min exposure period up to four times per day.

Subchronic: Intermediate between acute and chronic toxicities; subchronic toxicity studies involve repeated daily exposures of animals to a chemical for part (not exceeding 10%) of a lifespan. In rodents, this period extends up to 90 days exposure.

Susceptibility: Capacity to be adversely affected by pesticide treatment.

Synergism: An interaction of two or more chemicals that results in an effect that is greater than the sum of their effects taken independently.

Synthetic: man-made; produced by a synthesis of elements or materials, especially not of natural origin.

Systemic: Translocation. [Note (JWG): Acting throughout an organism or at a site different than that of exposure.]

$t_{1/2}$: Half-life.

Target organism: The target species is the organism which the pesticide is intended to control [or species at which a pesticide is directed by the method of application]. Conversely, non-target species are those which, because they are either beneficial or harmless, are not to be killed by the pesticide.

TD: Toxic dose; the dose of a chemical that produces signs of toxicity.

Teratogenic: A teratogenic agent has the ability to induce or increase the incidence of congenital malformations, i.e., permanent structural or functional deviations arising during embryogenesis.

Teratogenicity: The ability of a substance to produce irreversible birth defects or anatomical or functional disorders as a result of an effect on the developing embryo or fetus.

Theoretical Maximum Residue Contribution (TMRC): The amount of a substance that would be present in a 1.5 kg "average" daily diet if all commodities with established tolerances bore residues at the tolerance level. The percentages of various commodities in the "average" diet are those used by EPA and are based on the 1965-66 Household Food Consumption Survey conducted by USDA.

Threshold: The lowest dose of a chemical at which a specified measurable effect is observed and below which it is not observed.

Tissue: A group of similar cells.

TLm TL50: Median tolerance limit; for example, the concentration of chemical in water necessary to kill 50 percent of the test aquatic organisms during a specific exposure period. The TLm is usually expressed as parts per million parts of water for 24, 48, 72, or 96 hrs of exposure.

TLV: Threshold Limit Value; the highest allowable air concentration of a chemical in which workers may work for many years (8 hr/d, 5d/week, 50 weeks/yr) without negative health effects. Expressed as mg/m³; Threshold limit value-time weighted average. The time-weighted average concentration for a normal 8-hr workday and a 40-hr work week, to which nearly all workers may be repeatedly exposed without adverse effect.

Tolerance: (1) A legal limit, currently established by EPA, for the maximum amount of a pesticide residue which may be present in or on a food. Pesticide tolerances on raw agricultural commodities are listed in Part 180, CFR 40; tolerances on processed foods are listed in Part 193, CFR 21; and tolerances on processed animal feeds are listed in Part 561, CFR 21. Temporary tolerances, which cover residues resulting from an experimental use, generally expire after one year. (2) Capacity to withstand pesticide treatment without adverse effects on normal growth and function.

Toxic: Harmful; poisonous.

Toxicity: (1) The capacity or property of a substance to cause any adverse effects. It is based on scientifically verifiable data from animal or human exposure tests. (2) That specific quantity of a substance which may be expected, under specific conditions, to do damage to a specific living organism.

Toxicology: The study and or description of the toxicity of a active ingredient to organisms.

Transformation: Acquisition by a cell of the property of uncontrolled growth. [Note (JWG): More generally the change of a cell genetically in a manner permitting replication of the acquired characteristic, which usually comes from genetic material introduced into the cell, say by a virus.]

Translocation: Transport of a substance through a plant from the site of absorption to other portions of the plant.

Tumor: An unregulated overgrowth of cells.

TWA: The time-weighted average concentration is the average exposure concentration based on the duration of exposure to airborne concentration as it varies during an 8-hr workday.

Uncertainty factor: A number (equal to or greater than one) used to divide NOAEL or LOAEL values derived from measurements in animals or small groups of humans, in order to estimate a NOAEL value for the whole human population; also called margin-of-safety [or safety factor].

Vapor pressure: A relative measure of the volatility of a chemical in its pure state. The pressure exerted by a gas that is in equilibrium with its solid or liquid form [at a specified temperature]. Vapor pressure is a physical end point useful for understanding the distribution of the active ingredient between water/soil and air. High volatility is an indication of potential impact in the air compartment. This endpoint is determined for the active ingredient in a product and is typically reported as mm mercury (Hg) at a specified temperature.

Volatile: Capable of vaporizing or evaporating readily.

VSD: Virtually safe dose.

Water solubility: The maximum concentration of a chemical that dissolves in pure water at a specific temperature and pH.

WHO: World Health Organization.

Conversion Tables

Equivalent Measures of Length	
1 meter (m)	39.37 inches (in.)
1 centimeter (cm)	0.39 in.
1 millimeter (mm)	0.039 in.
1 yard (yd)	91.44 centimeters (cm)
1 foot (ft)	30.48 cm
1 inch (in.)	2.54 cm
Weight and Apothecaries' Equivalents	
1 milligram (mg)	1/65 grain (1/60)
1 gram (g)	15.43 grains (15)
1 kilogram (kg)	2.20 pounds
1 pound (avoirdupois)	453.6 grams
1 grain (gr)	0.065 gram (60 mg)
1 ounce (1/16 pound)	28.4 grams
Fluid Equivalents	
1 fluid ounce (oz.)	29.57 mL (30)
1 pint (pt.) 16 fl. oz.	473.2 mL (500)
1 pint, In the Imperial system	20 fluid ounces
1 quart (qt.)	946.4 mL (1000)
1 quart, In the Imperial system	40 fluid ounces
1 gallon (gal.)	3785.6 mL (4000)
1 gallon, In the Imperial system	160 fluid ounces
Metric System Weights and Measures	
1 kilogram (kg)	1000 grams (10^3)
1 milligram (mg)	0.001 gram (10^{-3})
1 microgram (μg)	0.000001 gram (10^{-6})
1 liter (L)	1000 mL (10^3)
1 milliliter (mL)	0.001 L (10^{-3})
1 microliter (μL)	0.000001 L (10^{-6})
1 milliliter (ml)	1 cubic centimeter (cc)
Weight - Unit Conversion Factors	

Units Given	Units Wanted	For Conversion Multiply By
lb	g	453.6
lb	kg	0.4536
oz	g	28.35
kg	lb	2.2046
kg	mg	1,000,000
kg	g	1,000
g	mg	1,000
g	μg	1,000,000
mg	μg	1,000
mg/g	mgL-1b	453.6
mg/kg	mgL-1b	0.4536
μg/kg	μgL-1b	0.4536
Mcal	kcal	1,000
kcal/kg	kcalL-1b	0.4536
kcalL-1b	kcal/kg	2.2046
ppm	μg/g	1
ppm	mg/kg	1
ppm	mgL-1b	0.4536
mg/kg	%	0.0001
ppm	%	0.0001
mg/g	%	0.1
g/kg	%	0.1

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