Transactions of the American Fisheries Society
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/utaf20

Issues Regarding the Use of Sedatives in Fisheries and the Need for Immediate-Release Options

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Published online: 12 Dec 2012.

To cite this article: J. T. Trushenski , J. D. Bowker , S. J. Cooke , D. Erdahl , T. Bell , J. R. MacMillan , R. P. Yanong , J. E. Hill , M. C. Fabrizio , J. E. Garvey & S. Sharon (2013) Issues Regarding the Use of Sedatives in Fisheries and the Need for Immediate-Release Options, Transactions of the American Fisheries Society, 142:1, 156-170, DOI: 10.1080/00028487.2012.732651

To link to this article: http://dx.doi.org/10.1080/00028487.2012.732651

Transactions of the American Fisheries Society 142:156–170, 2013

ISSN: 0002-8487 print / 1548-8659 online
DOI: 10.1080/00028487.2012.732651

FORUM

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Abstract

The lack of an immediate-release sedative (i.e., one for which no postsedation holding or withdrawal period is required) jeopardizes fish and fisheries research and poses considerable risk to those involved in aquatic resource

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Received March 29, 2012; accepted September 14, 2012
Published online December 12, 2012

management and the operation of public hatcheries and commercial fish farms. Carbon dioxide may be used as an immediate-release sedative, but it is slow-acting and difficult to apply uniformly and effectively. Tricaine methanesulfonate (MS-222) is easier to apply but requires a 21-d withdrawal period. The lack of an immediate-release sedative approved by the U.S. Food and Drug Administration (FDA) is a consequence of numerous factors, including the complexities of the approval process, the substantial human and monetary resources involved, and the specialized nature of the work. Efforts are currently underway to demonstrate the safety and effectiveness of benzocaine- and eugenol-based products as immediate-release sedatives. However, pursuing approvals within the current framework will consume an exorbitant amount of public and private resources and will take years to complete, even though both compounds are "generally recognized as safe" for certain applications by the FDA. We recommend using risk management–based approaches to increase the efficiency of the drug approval process and the availability of safe and effective drugs, including immediate-release sedatives, for use in the fisheries and aquaculture disciplines.

Access to safe and effective fish sedatives is a critical need of fisheries researchers, managers, and culturists. Federal, state, private, tribal, and academic fisheries professionals routinely sedate[1] fish for transport (e.g., moving them to a captive holding facility, stocking site, or to market), the collection of tissue samples (e.g., scales, spines, gametes, and fin clips) or morphometric data (e.g., length and weight), and the surgical implantation of tags or tracking devices (e.g., for monitoring movement, spawning behavior, or survival). Ideally, a fish sedative will be easy to administer, safe to use, and effective at low doses; provide quick and predictable sedation; offer some analgesia; elicit a state of sedation that is easily managed; have a reasonable margin of safety with respect to oversedation; be usable over a broad range of water chemistries; allow for rapid recovery from sedation and the physiological responses to the sedative; and be inexpensive. Additionally, it is often desirable that the sedative have no withdrawal period, meaning that sedated fish can be immediately released into the wild or taken to market upon recovery (typically referred to as "zero withdrawal" or "immediate release"). Unfortunately, there are few fish sedatives that possess all of these qualities, and at this time there are none that can be legally used in North America without a lengthy withdrawal period. Our objectives were to review the need for immediate-release sedatives, describe the current regulatory process for making such compounds available to fisheries professionals in North America, assess the relative risks associated with the use of two candidate immediate-release sedatives (a benzocaine-based product and a eugenol-based product), and provide recommendations to increase "regulatory efficiency" in the area of aquatic animal drug approvals as they pertain to fish sedatives. Specifically, we recommend a risk management–based approach to regulating the candidate immediate-release sedatives and outline a semiquantitative risk assessment which indicates that the proposed uses of these compounds have negligible risk.

[1]As discussed by Trushenski et al. (2012), the terms "anesthesia," "sedation," and "immobilization" are used somewhat interchangeably in fisheries science, but they actually have distinct definitions. Anesthesia is "a reversible, generalized loss of sensory perception accompanied by a sleep-like state induced by drugs or by physical means"; sedation is "a preliminary level of anesthesia, in which the response to stimulation is greatly reduced and some analgesia is achieved but sensory abilities are generally intact and loss of equilibrium does not occur"; and immobilization generally means the prevention of movement only (Ross and Ross 2008). Although these different definitions may be appropriate under different circumstances, most of the scenarios described herein are best described by the terms "sedate," "sedation," and "sedative"; for simplicity, we have used these terms throughout.

GENERAL NEED FOR SEDATION WHEN HANDLING FISH

Unlike most terrestrial vertebrates, which can be handled without causing significant mechanical damage, fish are particularly vulnerable to external and internal injury during physical restraint. Compared with the epithelium of terrestrial vertebrates, that of most fishes is delicate and prone to damage. The epithelium can be damaged by simply disrupting the protective mucus layer, potentially compromising osmoregulation and predisposing the fish to infection or infestation (Shephard 1994). Fish are innately difficult to handle, and when they actively resist restraint, epithelial damage or other physical injury to the fish or the handler is more likely. If fish are sedated prior to handling, the risk to both fish and handler is greatly minimized.

In addition to suffering mechanical damage, fish handled without proper sedation may be physiologically compromised as a result of stress. Stress may be defined as a natural reaction to a negative stimulus culminating in the mobilization and redirection of energy to support the "fight or flight" response (Selye 1950). During the stress response, the maintenance of important but not immediately critical functions is often sacrificed as a consequence of stress hormone release (Barton and Iwama 1991; Barton 2002). In fish, noncritical functions can include osmoregulation, reproduction, feeding, and particularly the exclusion and/or clearance of pathogens (Tort et al. 2004). As a result, stressed individuals may become homeostatically compromised and suffer tertiary consequences of stress, such as increased vulnerability to disease, reduced reproductive performance, and reduced growth (Barton and Iwama 1991; Wendelaar Bonga 1997; Barton 2002; Tort et al. 2004).

Beyond the readily quantified physiological consequences of handling unsedated fish, fisheries professionals must consider

TABLE 1. Attributes of currently available sedatives.

Sedative	Approved?	Limitations
Benzocaine	No, but can be used under INAD[a] authorization	3-d withdrawal period
CO_2	No, but FDA[b] unlikely to use regulatory authority	Cumbersome and not all fish respond well
Eugenol	No, but can be used under INAD authorization	3-d withdrawal period
MS-222	Yes for temporary immobilization	21-d withdrawal period

[a]Investigational New Animal Drug.
[b]U.S. Food and Drug Administration.

animal welfare (Huntingford et al. 2006). There is considerable scientific debate as to whether fish are capable of feeling pain or only exhibit nociception[2] (e.g., Rose 2002, 2003; Chandroo et al. 2004; Sneddon 2006); the specifics of this debate and its resolution are largely outside the scope of the present review. Regardless of whether fish perceive pain in the same manner as higher vertebrates, with respect to fisheries research, relevant guidelines advise that "investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals" (USPHS 1986; CCAC 2005), "prolonged stressful restraint [without appropriate sedation or anesthesia] should be avoided" (UFR 2004), and "procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia" (USPHS 1986).

CURRENTLY AVAILABLE SEDATIVES AND THEIR LIMITATIONS

Currently, there are few sedative options available to fisheries professionals that are safe, effective, and practical to use (Table 1). Perhaps more importantly, MS-222 (tricaine methanesulfonate [3-aminobenzoic acid ethyl ester methanesulfonate]) is the only compound approved by the U.S. Food and Drug Administration (FDA) and Health Canada for such use in these countries. Two MS-222 products (Tricaine-S and Finquel) are approved in the United States for the temporary immobilization of fish and other aquatic, cold-blooded animals, and one MS-222 product (Aqualife TMS) is approved in Canada for veterinary use only for anesthesia or the sedation of salmonids.

Like other local anesthetics, MS-222 is rapidly absorbed through the gills and believed to exert its sedative effect by preventing the generation and conduction of nerve impulses (Frazier and Narahashi 1975), though there is some uncertainty regarding this (Popovic et al. 2012). MS-222 has direct actions on the central nervous system, cardiovascular system, neuromuscular junctions, and ganglion synapses. Lower doses induce tranquilization and sedation, and higher doses result in general/surgical anesthetic planes (Alpharma 2001). In fish, brief tachycardia (elevated heart rate) occurs within 30 s of exposure, followed by prolonged bradycardia (depressed heart rate; Popovic et al. 2012). MS-222 also causes vasoconstriction in the gills, which slows down the uptake of waterborne materials across the gill membrane (Hunn and Allen 1974). Other effects of prolonged exposure include hypoxia (inadequate oxygen supply at the tissue or whole-body level), increased plasma lactate concentrations, hyperglycemia (elevated blood glucose levels), increased urinary output, and electrolyte loss. MS-222 is continually absorbed throughout immersion in spite of gill vasoconstriction and therefore can lead to a lethal overdose (Treves-Brown 2000). Additionally, unbuffered MS-222 acidifies water, and without sufficient buffering it may be stressful to fish (Popovic et al. 2012). Use of any pH buffering compound (e.g., sodium bicarbonate), however, adds an additional unapproved substance to the sedative solution. Despite its acidifying property, MS-222 is generally considered to be a safe and effective fish sedative and is widely used by fisheries professionals for a variety of purposes. According to the FDA product label instructions, the use of MS-222 should be restricted to fish in the ictalurid, salmonid, esocid, and percid families at water temperatures exceeding 10°C. In addition, a 21-d withdrawal period is required for use on fish intended for human consumption or fish that may be captured and consumed. The use of MS-222 is similarly restricted in Canada, including a provision that treated fish cannot be slaughtered until 5 d after the last exposure. Also, during this holding period, fish must be held in water warmer than 10°C (Health Canada 2010). For many applications, holding fish for 5 d postsedation is not practical or seriously compromises the objectives of management or research activities. In field settings, it can be extremely problematic to hold fish for as little as 1–2 h posttreatment without utilizing specialized equipment and allotting additional time to complete such field procedures. Most severely affected are fisheries professionals collecting population morphometric data or gametes from wild-caught fish and those involved in surgically implanting devices (e.g., electronic or acoustic tags) in catchable-sized fish. To avoid the complications of holding fish in field settings, an approved immediate-release sedative is critically needed.

Currently, the only immediate-release sedative compound available in the United States is CO_2, which is considered a

[2]As discussed by Sneddon (2009), the generally accepted definition of "pain" involves two elements: (1) the perception of stimuli associated with actual or potential tissue damage, referred to as nociception; and (2) awareness of an associated negative emotional experience, sometimes described as discomfort or suffering. It is relatively easy to demonstrate nociception in fish. However, it is impossible to demonstrate what a fish "feels" and therefore whether it can experience pain as it is defined.

low regulatory priority (LRP) drug by the FDA[3]. Although CO_2 gas has been characterized by some as an effective sedative (primarily for freshwater fishes), it is generally not considered safe for target animals because it is slow-acting and difficult to apply uniformly and often results in adverse outcomes, including presedation hyperactivity and postsedation morbidity and mortality. There is a large body of research on the physiological consequences of hypercapnia in fish (e.g., Tufts and Perry 1998) indicating that CO_2 gas is not an ideal sedative.

Sedative concentrations of CO_2 can be established in two primary ways: CO_2 gas can be bubbled into the water until the desired concentration is achieved, or CO_2 can be produced by the addition of sodium bicarbonate to acidified water. In each of these cases, the concentration of CO_2 must be closely monitored to achieve and maintain the target concentrations (e.g., Trushenski et al. 2012). Compared with other sedatives, CO_2 can be logistically difficult to use because it requires continuous monitoring and the adjustment of concentrations and it may require the use of heavy, bulky, and potentially hazardous pressurized gas cylinders.

The effectiveness of CO_2 as a sedative is based on its interference with normal respiratory exchange. Under normal conditions, CO_2 produced by a fish's tissues is transported via the circulatory system to the gill, where it is excreted via diffusion down the blood–water tension gradient. When environmental concentrations of CO_2 are high, this process is slowed or reversed, causing CO_2 to build up in the bloodstream and tissues (Perry et al. 2009). When CO_2 is applied as a sedative, respiratory levels of the gas build in the central nervous system, interfering with the normal metabolism and function of these cells. Gradually, widespread central nervous system depression occurs, resulting in the loss of consciousness and voluntary motor function, though involuntary movements may occur in CO_2-sedated fish (Iwama and Ackerman 1994).

Induction times for CO_2 are usually long (Trushenski et al. 2012) and are typically accompanied by a period of intense hyperactivity. A short period of hyperactivity is commonly observed during the induction phase of sedation, likely due to the presence and irritating nature of sedatives (Ross and Ross 2008). However, the hyperactive response to CO_2 may be more pronounced in some species, which exhibit strong avoidance behaviors upon exposure to sedative concentrations of CO_2 (e.g., Bernier and Randall [1998] observed fish "violently struggling") and may remain agitated for extended periods of time (minutes) before passing into the early stages of sedation. Recovery times following CO_2 sedation are also typically extended, greatly exceeding the ideal time frames for fish sedatives.

To sedate fish with CO_2, hypercapnia must be induced. Although this achieves the desired result in terms of sedation, hypercapnia affects all major organ systems and considerable time is needed to fully compensate for the resulting acidosis. It is perhaps not surprising that exposure to elevated environmental CO_2 also induces the generalized stress response in fish and can result in direct or delayed mortality if exposure concentrations or durations are excessive. Thus, depending on the duration and severity of the response, exposed fish will experience the consequences of corticosteroid or catecholamine release (Barton and Iwama 1991). The direct and indirect (via actions of corticosteroid/catecholamine release) effects of CO_2 exposure include acid–base disruption and lactate accumulation, osmoregulatory dysfunction, and elevated plasma glucose (Trushenski et al. 2012). Depending on exposure conditions, full recovery from these disturbances can take hours or days (Wagner et al. 2002; Pirhonen and Schreck 2003).

Although the sedative application of CO_2 can be problematic, if it is applied correctly, exposure often results in the light sedation and immobilization of many freshwater species. The same cannot be said for marine species, however; because of the high concentration of ions in the marine environment, the solubility of CO_2 is reduced, making it more difficult to achieve sedative concentrations of CO_2 in brackish or saltwater. Additionally, CO_2 excretion occurs more readily in marine environs, making it difficult to induce hypercapnia in marine species. In some situations, achieving the desired levels of sedation in marine species with CO_2 requires decreasing water pH to 5–6, which can have unintended negative consequences (e.g., morbidity and mortality; R. P. Yanong, unpublished data); in a recent study assessing the light sedation of Cobia *Rachycentron canadum*, we demonstrated that CO_2 decreased the pH of brackish water (20‰) with 88 mg $CaCO_3$/L alkalinity by more than one unit (Trushenski et al., in press); similar results were reported for high-alkalinity (>200 mg $CaCO_3$/L) seawater CO_2 sedation baths used in the harvest and slaughter of Atlantic Salmon *Salmo salar* (Erikson 2008). Because CO_2 can be impractical for field use, typically allows for only light sedation, can induce long-term physiological disruptions, and is not fully appropriate for marine species, it is not considered a suitable sedative by the majority of fisheries professionals.

Neither MS-222 nor CO_2 is a viable option for broad use as a fish sedative in field situations. Similarly, hatchery personnel who wish to lightly sedate fish to improve the poststocking survival of catchable-size fish by reducing stress during transport have no reasonable options. As a result, fisheries professionals are often faced with a difficult choice—to use MS-222 off-label (i.e., disregarding the 21-d withdrawal period), to use unapproved sedative compounds, or to use nothing at all. In the United States, off-label drug use and the use of unapproved drugs are both illegal. Although the Animal Medicinal Drug Use Clarification Act (AMDUCA) allows some extra-label drug use with veterinary oversight, such use is limited to circumstances "when the health of an animal is threatened or suffering or death may result from failure to treat" (USOFR 2002a).

[3]For the drugs in this category, the FDA has determined that regulatory action is unlikely as long as an appropriate grade is used, they are used for the listed indication at the prescribed levels, good management practices are followed, and local environmental requirements are met (USFDA 2011c).

Although sedatives are often associated with applications focusing on animal health and well-being, these uses are likely outside the intended scope of AMDUCA. Hence, the lack of an approved immediate-release sedative that is safe and effective presents fisheries professionals with both a legal and an ethical dilemma: They must (1) adhere to their individual ethics and the guidelines established for the fisheries profession and treat fish humanely with safe and effective (albeit unapproved) sedatives prior to procedures causing distress, (2) use FDA-approved or LRP drugs according to the label instructions and be severely constrained by impractical withdrawal periods or the risk of harming fish during sedation, or (3) use nothing and defy the spirit of all relevant animal welfare regulations and guidance documents.

A widely used set of guidelines for the use of fish in research published jointly by the American Fisheries Society (AFS), the American Institute of Fishery Research Biologists, and the American Society of Ichthyologists and Herpetologists (UFR 2004) states that "prolonged stressful restraint [without appropriate sedation or anesthesia] should be avoided" but also stipulates that

> the full range of potential effects on the subject fish, not just the sedative qualities, must be considered. The sedative chosen should be one that permits a rapid return to normal physiological and behavioral status and is a low risk compound for humans as well as fish.

The use of sedatives in fisheries work is also described in two seminal AFS publications, *Fisheries Techniques* (Murphy and Willis 1996) and *Methods for Fish Biology* (Schreck and Moyle 1990), which advocate using sedatives as a routine part of fish care and handling (Kelsch and Shields 1996) and provide detailed explanations of sedatives and their use (Summerfelt and Smith 1990). It is imperative that a practical, safe, effective, and approved sedative be available to conform to the guidance provided by such documents, but the options are severely limited. Conducting procedures that cause distress without proper sedation/anesthesia is not appropriate from the perspective of animal welfare; poses risk to personnel (particularly in the case of large fish or fish that are otherwise hazardous when handled without restraint) and the animals themselves; and is not consistent with the spirit and recommendations of any of the aforementioned guidance documents.

PROCESS FOR GAINING AQUATIC ANIMAL DRUG APPROVALS AND THE CURRENT REGULATORY ENVIRONMENT: THE U.S. EXPERIENCE

The pursuit of FDA approval of an immediate-release sedative has been long and, to date, fruitless. This is a consequence of numerous factors, including the complexities of the drug approval process, the substantial human and monetary resources which must be expended in pursuit of an approval, the low potential for return on investment by pharmaceutical firms, the specialized nature of the work, and the limited number of personnel and institutions engaged in drug approval research and support activities.

Under authority of the Federal Food, Drug, and Cosmetic Act (FFDCA), the FDA's Center for Veterinary Medicine (CVM) regulates the manufacture, distribution, approval, and use of animal drugs. This regulatory authority includes drugs for use in food-producing animals such as fish as well as pets/companion animals. With respect to drugs that are used in food-producing animals, the CVM is responsible for ensuring that the drugs are safe and effective and that the food products derived from treated animals are free from potentially harmful drug residues.

What Qualifies as a Drug?

A drug is defined by FDA as

> any article that is intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; any article (other than food) intended to affect the structure or function of the body of man or other animals; or any article that is recognized in official drug compendia (USC 2010).

This definition is extremely broad and would apply to any substance other than the unadulterated food fed to a fish and the unadulterated water in which fish live, including virtually any compound administered to fish via immersion, feed, injection, or any other method. The breadth of this definition is clearly exemplified by the CVM's List of Drugs of Low Regulatory Priority, which includes, among other compounds, onion and garlic to control or reduce infestations of some ectoparasites, salt (NaCl) for osmoregulation or as a parasiticide, ice to reduce the metabolic rate of fish during transport, and fuller's earth to reduce the adhesiveness of fish eggs. More recently, the CVM has suggested that pre- or probiotics derived from the natural gut microflora of fish may be classified as drugs if advertised (or promoted) as such, i.e., to control or reduce an infectious fish pathogen, disease symptom, or mortality.

What Qualifies as a Food Fish?

Although the CVM has not clearly defined food fish in laws or regulations, the general consensus is that the agency considers *any* fish that is potentially available for human consumption to be a food fish—a very broad definition (USFDA 2008). Obviously, fish raised commercially for sale as live, in-the-round, filleted, or otherwise processed products are considered food fish. Less obvious is the fact that fish released for restoration/recovery, stock enhancement, mitigation, recreational fishing, or other management purposes are also generally considered food fish. The rationale is that if at any point in time hatchery-raised fish are available for legal harvest (i.e., angling or commercial fishing), they are potentially available for human consumption and are therefore food fish. Threatened or endangered species which cannot be legally harvested are not considered food fish by the CVM; however, it is unclear whether this rationale extends to sublegal life stages of game fishes or nongame fishes. Although common sense suggests that sublegal and nongame

fishes would not be considered harvestable or consumable, this does not appear to be the case. Even more ambiguous is the status of baitfish, which may be indirectly incorporated into the food chain via the absorption by game fish of drug residues from baitfish consumed naturally or as a result of angling activity. Although the CVM has published a guidance regarding this issue, it is relatively vague and does not agree with some recent decisions (USFDA 2008). For example, the CVM has determined that "feeder" goldfish (i.e., goldfish sold to hobbyists, aquariums, zoos, and others as live food for other animals) are considered food fish. Until more definitive guidance is issued, it would seem prudent to assume that all nonimperiled fish species are considered food fish, with the possible exception of certain "ornamental and aquarium fish" strictly associated with the aquarium trade (USFDA 2008).

Data Requirements and Other Challenges to Gaining Approvals

The CVM approves new animal drugs based on review of the data submitted by the pharmaceutical or chemical company drug sponsor. In the case of aquaculture drugs, for which the economic incentives for pharmaceutical sponsors to seek a new approval are typically extremely low, sponsor-submitted data are augmented by data generated by public sector entities (e.g., the U.S. Fish and Wildlife Service [USFWS], the U.S. Geological Survey [USGS], the Agricultural Research Service of the U.S. Department of Agriculture [USDA–ARS], the CVM's Office of Research, and several universities). When all data have been accepted by the CVM, the sponsor requests a new drug approval via a new animal drug application (NADA). The data required for approval must demonstrate that the drug is effective as claimed on the label and safe when used as directed for (1) the treated animals, (2) the people administering the treatment, (3) the environment, including nontarget organisms, and (4) consumers. To demonstrate the efficacy and safety of a specific drug, a drug sponsor must adequately address eight NADA technical sections (information categories): (1) chemistry, manufacturing, and controls, (2) human food safety, (3) effectiveness, (4) target animal safety, (5) environmental safety, (6) labeling, (7) freedom of information summary, and (8) all other information.

For any animal species, the successful completion of all NADA technical section requirements is time-consuming and expensive. Previous reports have suggested that getting a new aquaculture drug approved required a minimum investment of $3.5 million (Schnick et al. 1996) over the course of a decade. More recent estimates indicate that new drug approvals may cost in excess of $40 million, and label expansions to include new species or claims may cost as much as $8 million (Storey 2005). In some respects, new drug approvals for fish are more difficult to obtain than new drug approvals for virtually any terrestrial animal. Unlike companion animals, fish are considered a potential source of food for humans, and fish drug approvals require completion of the full tier of studies to satisfy the human food safety technical section. Of all the data requirements, the human food safety technical section is typically the most expensive to complete. Completion of this section is also required for terrestrial livestock drugs, but with respect to NADA data requirements the CVM views fish differently than it does other food or companion animals. Although the CVM considers all breeds of a single species to represent the species, it does not consider any fish species to be representative of fish collectively or categorically. Although the CVM will accept studies conducted on a single cattle or dog breed as sufficient to complete the NADA technical sections for all cattle or dog breeds, studies conducted on a single fish species generally apply to that species only. Hundreds of freshwater fish species and varieties are currently raised in the United States, resulting in significant implications of this "species-by-species" approach to data requirements. Although the CVM has allowed species grouping for drug approvals for freshwater fish (i.e., data from two or more species within a group of fish are considered sufficient for all group members, e.g., all freshwater salmonid species), the data requirements for "all fish" label claims remain significantly greater than similar claims for other animal groups. The additional NADA data requirements associated with marine fish drug approvals have not yet been fully explored, but given the potential differences between marine and freshwater species and the conditions in marine and freshwater environments, it is anticipated that the data requirements for label expansion to marine species will be significant.

Because of low economic incentives and high regulatory requirements for aquaculture drugs, drug sponsors have relied heavily on assistance from public entities to complete NADA requirements. Public data-generating partners comprise a small but dedicated cadre of member agencies constituting the Federal–State Drug Approval Partnership Project (PROJECT) under the direction of the Association of Fish and Wildlife Agencies (AFWA). The PROJECT was established in 1995 to work collaboratively toward the approval of eight priority aquaculture drugs and continues such efforts today. Oversight of the PROJECT is provided by the Drug Approval Working Group (DAWG), which was formed in 1997 under the Fisheries and Water Resources Policy Committee of the AFWA. In addition to the members representing the AFWA (the 50 state fish and game agencies), DAWG members from the USFWS, USGS, and USDA–ARS help to coordinate aquatic drug approval activities and set PROJECT priorities. While initial approvals for four of the PROJECT drugs have been obtained, these approvals have been restricted to use in certain species (or species groups) and/or to control mortality caused by specific pathogens. Seventeen years after the PROJECT was formed, the other priority drugs still await initial approvals. Obtaining an initial approval for an immediate-release field sedative remains the DAWG's highest priority, with approval for all freshwater finfish as the principal goal.

While the DAWG and its technical partners have made significant progress and are committed to pursuing new and/or expanded aquatic species drug approvals, progress has been slow. Dedicated resources (i.e., funding and staff with sufficient expertise to develop study protocols acceptable to the CVM and conduct studies according to good clinical practices or in compliance with good laboratory practices [GLP] regulations) are simply insufficient to accomplish the work in a timely manner. This situation has been exacerbated by changing program priorities among the public data-generating partners that have resulted in erosion of the resources dedicated to the fish drug approval process. For example, in 2011 the USGS eliminated all drug approval research funding from its budget. Today the USFWS's Aquatic Animal Drug Approval Program is the only program comprising more than one senior scientist with a full-time commitment to aquatic species drug approval efforts. Other entities continue to contribute to the process (e.g., the CVM's Office of Research, National Research Support Program-7, Mississippi State University, the University of Idaho, Auburn University, and others), but the available resources simply do not meet needs. However, current difficulties are not solely related to the lack of sufficient financial resources. The limited number of institutions and individuals capable of, and interested in, completing the needed work is an equally important constraint. Many of the studies required to gain FDA approval of a drug require that the testing be conducted at facilities that comply with GLP regulations; few fisheries institutions are capable of meeting these standards or are willing to do so. The regulatory process for aquatic species drug approvals is inherently arduous—as it should be—to ensure public health and safety; however, the current regulatory process and atmosphere may be more restrictive than necessary or appropriate. In short, aquatic species drug approval efforts are challenged by low economic incentives for sponsors (often small chemical companies with minimal budgets for research and development), a relatively small group of active participants, and a very expensive and arduous regulatory process.

Previous Attempts to Gain Approval for an Immediate-Release Sedative

In the past, several compounds have been considered as candidate immediate-release sedatives, including MS-222, benzocaine, and isoeugenol. Originally, it was thought that expanding the current MS-222 approval to reduce the 21-d withdrawal period (which, to the best of our knowledge, was somewhat arbitrarily defined by the CVM) or obtaining new animal drug approvals for the other two products would occur in a timely manner. However, pursuit of an immediate-release approval reached an impasse for each of these compounds. The sponsors for MS-222 do not wish to devote the necessary resources to provide the FDA with data to support the immediate-release label claims for their products. Furthermore, these sponsors were justifiably concerned that efforts to modify current labels might jeopardize their existing approvals. Requests for label expansions allow the CVM to revisit and reassess previously submitted data. Given the changes to the regulatory landscape, it is plausible that CVM reviewers would deem previously accepted data insufficient to meet current requirements, rendering existing approvals invalid. This concern, whether real or perceived, is warranted because over time the FDA has significantly "raised the bar" relative to the data required to support approvals. The approval guidelines and criteria in place when MS-222 was approved were significantly less rigorous than those used for multispecies drug approvals today. Thus, without sponsor support, it was concluded that MS-222 is a nonstarter for immediate-release uses. In 1994, benzocaine was identified as an immediate-release fish sedative candidate. Unfortunately, the approval process for benzocaine was not pursued because no drug company was willing to sponsor such efforts. As a result, in 1996, the focus of approval efforts shifted to AQUI-S (50% isoeugenol [4-propenyl-2-methoxyphenol]), which had existing immediate-release approvals in several other countries (Australia, Chile, New Zealand, and others), and a committed sponsor (AQUI-S New Zealand, Ltd., Lower Hutt, New Zealand) willing to pursue FDA approval. During the ensuing 10 years, significant progress was made toward assembling an AQUI-S NADA package in support of an immediate-release claim for all freshwater finfish. AQUI-S was on the home stretch for an initial FDA approval, but due to unforeseen potential human health concerns all research was permanently derailed in April–May 2007. Reviewers of a 2-year cancer study on rats and mice (conducted by the National Toxicology Program [NTP]) concluded that isoeugenol demonstrated "some" level of carcinogenicity, and the FDA subsequently terminated further review of the data in support of an approval for AQUI-S (in accordance with the Delaney Clause of the FFDCA). Despite being generally recognized as safe and approved by the FDA for direct use in human foods (NTP 1991), the use of isoeugenol as a fish sedative was deemed to pose an unacceptable human food safety risk. Thus, in the United States, isoeugenol-based products (such as AQUI-S) or clove oil (which contains some isoeugenol) will likely never be approved for use as fish sedatives (USDHHS 2007). However, this finding will not affect previous FDA approvals for the use of isoeugenol in foods.

Interestingly, the European Medicines Agency (EMA), which regulates the use of animal drugs in the European Union (EU), reviewed the NTP isoeugenol carcinogenicity report and nonetheless licensed the product as a sedative in fishes (EMA 2008). More specifically, data from the report have been used to establish an allowable dietary intake of 0.0075 mg isoeugenol/kg body weight (~45 mg isoeugenol/person) based on a maximum residue level of 6,000 μg isoeugenol/kg fish tissue (muscle and skin in natural proportions; EMA 2011). This development will allow isoeugenol products to be registered in the EU. Additionally, in the EU a sponsor that wishes to market a new drug need only submit a single application to the EMA for a "marketing authorization" (license) that is valid in all EU member states as well as Iceland, Liechtenstein, and Norway.

CANDIDATE IMMEDIATE-RELEASE SEDATIVES AND THEIR EFFECTS

Efforts spearheaded and prioritized by the DAWG are currently underway to evaluate the safety and effectiveness of other candidate immediate-release fish sedatives. Currently, Benzoak (a benzocaine-based product) and AQUI-S 20E (a eugenol-based product manufactured by the producer of AQUI-S [which is isoeugenol based]) are the top candidates with engaged sponsors willing to pursue approvals.

Benzocaine

Benzocaine has been used as a topical pain reliever since the end of the 19th century. Benzocaine acts on the central nervous and cardiovascular systems, neuromuscular junctions, and ganglion synapses, eliciting sedative, anesthetic, and analgesic effects by interfering with the changes in membrane permeability necessary to conduct nervous stimuli. Benzocaine is chemically similar to MS-222 but relatively insoluble in water (McErlean and Kennedy 1968; Allen et al. 1994) and must be dissolved in alcohol or acetone prior to use. Once in solution, benzocaine has a neutral pH. As a result, benzocaine causes less hyperactivity than unbuffered MS-222 during induction, though buffered MS-222 is chemically similar to benzocaine and yields similar physiological responses (Ross and Ross 2008).

The sedative effect of benzocaine on fish was first reported by McErlean (1967) and McErlean and Kennedy (1968). Since then, extensive and ongoing research has demonstrated that benzocaine is effective for sedating a variety of freshwater and saltwater fishes under a wide range of environmental conditions (Laird and Oswald 1975; Ferreira et al. 1984; Marking and Meyer 1985; Gilderhus and Marking 1987; Gilderhus 1990; Gilderhus et al. 1991; Gomes et al. 2001; Iversen et al. 2003; Hasan and Bart 2007; Kiessling et al. 2009). Induction times are usually faster and recovery times usually slower for benzocaine-treated fish than for MS-222–treated fish (McErlean and Kennedy 1968; Ferreira et al. 1979; Gilderhus and Marking 1987; Mattson and Riple 1989; Munday and Wilson 1997; Kiessling et al. 2009). In general, benzocaine administered in a static bath at concentrations ranging from 25 to 150 mg/L effectively sedates a variety of freshwater and marine fishes.

Benzocaine is not toxic to humans at the concentrations suggested for sedating fish, and higher concentrations are routinely used in healthcare products for humans and food animals other than fish. Benzocaine is the active ingredient in nearly 100 over-the-counter topical analgesic products (e.g., oral pain relief products). In these products, benzocaine concentrations may be as high as 200,000 mg benzocaine/kg (20%). Although benzocaine can induce methemoglobinemia, a condition which interferes with the oxygen-carrying capacity of the blood, serious toxicity is extremely rare. It has been estimated that more than 1 million human exposures to benzocaine (intentional and accidental) occur every year in the United States, yet fewer than 100 cases of methemoglobinemia induced by over-the-counter benzocaine products have been reported in the medical literature over the last 50 years (Suchard and Rudkin 2004). Although additional cases have been reported, these were in association with the use of prescription benzocaine products by healthcare professionals in the course of medical procedures (USFDA 2011a) or the misuse of over-the-counter products in children (USFDA 2011b). Benzocaine is used in cattle, sheep, swine, and horses for local and prolonged low-epidural (without paralysis of the hind limbs) anesthesia and in ointments for minor wounds and ulcerated surfaces (EMA 2001). When used as a local anesthetic in livestock, concentrations ranging from 150 to 750 mg/animal are used, whereas topical ointments typically contain 5,000 mg benzocaine/kg (0.5%). In addition to being effective at much lower doses for the sedation of fish, benzocaine leaves very low incurred tissue residues in fish. Tissue residues in Rainbow Trout *Oncorhynchus mykiss* and Largemouth Bass *Micropterus salmoides* exposed to 50 mg/L benzocaine for 15 min were less than 15 μg/g immediately after treatment, and residues decreased to less than control values after 4 h for Rainbow Trout and after 8 h for Largemouth Bass (Allen 1988). In general, tissue levels of benzocaine decrease to undetectable levels within 2 h of exposure (Ross and Ross 2008).

Eugenol

Eugenol is a pale-yellow, oily compound derived from the flowers, stalks, and leaves of various plants, including clove *Syzygium aromaticum*, nutmeg *Myristica* spp., cinnamon *Cinnamomum* spp., basil *Ocimum basilicum*, and bay or laurel (Lauraceae and Myrtaceae families). Like benzocaine, eugenol elicits sedative, anesthetic, and analgesic effects by interfering with the changes in membrane permeability associated with the transmission of nervous signals. Eugenol is a phenylpropanoid, a class of chemicals that includes numerous plant-derived compounds involved in the defense against herbivory and other sources of plant injury. Long recognized for its anesthetic and antiseptic properties, eugenol is commonly used in dentistry for topical pain relief, as a local anesthetic, and in the preparation of temporary fillings. Because of its high eugenol content (85–95%), clove oil has been used since antiquity as a mild topical anesthetic for the treatment of toothaches, headaches, and joint pain (Ross and Ross 2008). Although eugenol exposure may cause gastrointestinal and cardiorespiratory symptoms and contact dermatitis in those with a eugenol allergy, clove oil and its derivatives (including eugenol) are generally recognized as safe in the United States for direct inclusion in food (USOFR 2002b). In addition, the Joint Food and Agriculture Organization (FAO)–World Health Organization (WHO) Expert Committees on Food Additives consider up to 2.5 mg · kg body weight^{-1} · d^{-1} an acceptable daily intake for humans (JECFA 2006).

The sedative effect of eugenol in fish was first reported in the early 1970s by Endo et al. (1972). However, little information is available for this compound because most of the research has focused on clove oil (as a crude product) or isoeugenol (as a derived product). For example, since 1995 more than 40 papers have been published on clove oil (Ross and Ross 2008)

summarizing its effectiveness in sedating a variety of fishes (e.g., Soto and Burhanuddin 1995; Taylor and Roberts 1999; Iversen et al. 2003; Park et al. 2008). Research on eugenol indicates that it is effective for sedating both freshwater and saltwater fishes (Hikasa et al. 1986; Roubach et al. 2005; Palić et al. 2006). Like isoeugenol, eugenol appears to depurate rapidly from fish tissues. The half-life of eugenol in the blood of Rainbow Trout exposed to a 75-mg/L solution for 15 min was estimated to be 12 h. Blood concentrations of eugenol declined to 8 μg/mL 1 h postexposure, and after 20 h blood levels were 0.1 μg/mL (Guénette et al. 2007).

Other Similar Sedatives

Carvone is the primary component of spearmint oil and gives the oil its minty fragrance and flavor. Methyl salicylate is a related compound which gives wintergreen oil its distinctive fragrance and flavor. Both spearmint and wintergreen oils are commonly used in foods, chewing gum, cosmetics, toothpaste, tobacco products, and pharmaceutical preparations (Chen et al. 2010). Preliminary research has shown that fish can be effectively sedated with a carvone–methyl salicylate emulsion (Danner et al. 2011). Like eugenol and benzocaine, compounds such as carvone and methyl salicylate would appear to be relatively innocuous and pose little, if any, safety risk to humans in the context of fish sedation. Nonetheless, these compounds would be subjected to the same drug approval process as eugenol, benzocaine, or more "pharmaceutical-type" drugs intended for use in fish.

END USERS AND DEMAND FOR IMMEDIATE-RELEASE SEDATIVES

Fisheries Management and Research

The North American model of fisheries management is "science based," meaning that research activities, stock assessments, and knowledge are the fundamental components of the decision-making process (Lackey 2005). Fish sedation in fisheries management is a part of applied research projects to assess the status of fish populations, evaluate critical habitat, and study fish movement, migration, and community connectivity. Associated with each of these types of research activities is a suite of tools and techniques that require fish to be temporarily sedated to protect them and the fisheries scientists. The three most common techniques in fisheries include basic enumeration and morphometrics, collection of biological samples, and marking or tagging of fish.

Routine tasks such as species identification, enumeration, and morphometric and meristic measurements (e.g., performing lateral line scale counts and determining the presence/absence of various morphological features) can be facilitated by the use of sedatives. The same is true for the weight and length measurements used to assess and monitor the growth and condition of fish and the direct or ultrasound examinations used to determine the sex and maturity of fish (e.g., Martin et al. 1983; Martin-Robichaud and Rommens 2001). These data are fundamental components of stock assessments and other fisheries research activities. If these data go uncollected or are biased because of an inability to handle fish properly, model outputs and subsequent conclusions and management decisions will be affected.

Fisheries research and management activities commonly involve the collection of biological samples such as scales, spines, or fin clips from live fish. These samples are used to assess fish age, the presence of genetic or other molecular markers, and other attributes used to determine life history, growth rate, population structure, habitat or food resource utilization, and other characteristics. Although collecting fin clips or scales is relatively quick and simple, it is greatly facilitated by sedation, especially when large or otherwise hazardous fish are involved. Collection of other samples requires more invasive techniques, so sedation is at least recommended and typically mandated by Institutional Animal Care and Use Committees. These activities may include blood sampling, gamete collection, muscle or liver biopsies, or spine extraction (Gilliland 1994; Grant 1996) or laparotomy (creating an incision in the body wall), endoscopy, or ultrasound procedures (Mattson 1991; Hurvitz et al. 2007), all of which require or greatly benefit from sedation.

Marking and tagging techniques, which are commonly used in estimating fish mortality or movement, often require sedation to facilitate fish handling and to fully comply with fish welfare guidelines (e.g., Mulcahy 2003). Simple marks or tags (e.g., fin clips and anchor tags) can be applied directly, but lightly sedating fish can improve tagging efficiency and "throughput" in the field. Other tags, such as telemetry tags (e.g., radio tags, PIT tags, and acoustic tags) and archival biologgers must be surgically implanted within the body cavity or anchored externally, intramuscularly, or gastrically; these more invasive procedures obviously require fish to be sedated prior to tagging (Mulcahy 2003; Cooke et al. 2011). Such tags have proven invaluable in studying the behavior and spatial ecology of fish (e.g., Lucas and Baras 2000) as well as their migratory behavior, site fidelity, habitat use, and home range size. Telemetry techniques are also being applied to the study of endangered or threatened fish species (Cooke 2008). Physiological and behavioral approaches are also being implemented in the field (reviewed by Cooke et al. 2004; Costa and Sinervo 2004; and Wikelski and Cooke 2006), aided by the use of telemetry and biologging techniques that incorporate a variety of specialized sensors (e.g., Lucas et al. 1993; Gillooly and Baylis 1999; Clark et al. 2009). These techniques are used throughout North America, and the number of studies employing them is increasing (Cooke et al. 2011). However, researchers are limited to the drugs that are legally available for these procedures (Jepsen et al. 2002), and currently available sedatives have serious limitations in terms of their unintended effects or regulatory compliance issues (Mulcahy 2003). For example, researchers using MS-222, eugenol, or benzocaine must hold fish for an extended period of time prior to release; in these circumstances, behavioral artifacts associated with holding animals in captivity will likely be introduced—exactly the type of effect

that field-based physiological studies are designed to avoid. Surgical tagging is regarded as a topic requiring further research and refinement (Cooke and Wagner 2004), and given the growing interest in using these techniques, it is reasonable to expect an increased need for immediate-release sedatives for this purpose.

Public Aquaculture

The U.S. and Canadian governments are major producers of cultured fish for recreational and nonconsumptive uses; in the United States, the majority of fisheries professionals employed by natural resources agencies in the fisheries-related disciplines are employed by state or federal hatcheries. Roughly 1.75 billion hatchery-raised fish were stocked in U.S. waters in 2004, including commercially and recreationally important species as well as imperiled fishes (Halverson 2008). In 2005, the number had increased to approximately 1.89 billion fish (USFWS 2006). Most, if not all, of these fish were handled and transported during rearing or stocking; much of this could have been accomplished more efficiently using sedatives. Sedation is also advantageous in the hatchery setting for the collection of gametes from wild-captured or captive broodstock. Although the use of CO_2 or MS-222 (with the appropriate 21-d withdrawal period) for transport, handling, or gamete collection might be feasible in some cases, in most circumstances neither sedative would be appropriate. Public hatchery systems are central to aquatic resources management in North America, and the lack of an immediate-release sedative complicates or threatens the ability of these operations to legally fulfill their obligations and meet aquatic resource management goals.

Private Aquaculture

In addition to the sizable commercial sport fish, bait, and ornamental fish industries, approximately 300,000 metric tons of food fish are raised annually in the United States (NMFS 2011). Aquaculture is a US$1 billion/year industry in the United States, providing a healthy source of protein as well as domestic jobs (NMFS 2011). However, domestic aquaculture production is dwarfed by imported seafood products: in 2010, the United States imported more than 5.5 billion pounds of edible seafood products valued at $14.8 billion and contributing to an annual seafood deficit of more than $10 billion. Eighty-six percent of the seafood consumed in the United States is imported, and of these imported products approximately 50% are farm-raised (NMFS 2011). Demand for sedatives at commercial farms is similar to that at public hatcheries, including the need to sedate broodstock and facilitate the movement of fish within a facility and the transport of fish to other culture facilities or to market. Ironically, many of the countries from which we import seafood have access to immediate-release sedatives.

Summary of Demand for Immediate-Release Sedatives

The various endeavors outlined in this section have significant economic, ecological, and scientific value that is threatened by the lack of an approved immediate-release sedative. Public aquaculture is instrumental in maintaining commercial and recreational fishing opportunities in North American waters, and conservation aquaculture is central to efforts to restore imperiled fish species. In the case of private aquaculture, the FAO estimates that for each individual employed directly in the aquaculture industry, up to four more individuals are supported indirectly in the processing, marketing, and other associated industries; as much as 8% of the world's population depends on aquaculture for part or all of their livelihood (FAO 2008). Although less readily quantified in a tangible, monetary sense, accurate data are invaluable to fisheries research efforts, as are the outcomes these data yield when translated into practice and policy in the fisheries disciplines.

OTHER PRACTICAL CONSIDERATIONS

Approvals in Other Countries

Very few sedatives are approved for use in fish in other countries, and only two (eugenol and isoeugenol) are approved as immediate-release sedatives. Whether this reflects low demand for sedatives, the use of sedatives under veterinary discretion, or illegal use is unknown. MS-222 is the most widely used fish sedative in the world. This sedative is approved for food fish use in the United States, Canada, and Europe (Popovic et al. 2012) as well as in Australia and New Zealand. All countries with MS-222 approvals appear to require a 5- or 21-d withdrawal period. Metomidate [1-(1-phenylethyl)-1H-imidazoel-5-carboxylic acid methyl ester] is approved for use in Canada (Schnick 2001) and is "Indexed" in the United States (i.e., as an unapproved drug that can be legally marketed for ornamental aquarium fish); chlorobutanol [1,1,1-trichloro-2-methyl-2-propanol] and benzocaine [p-aminobenzoic acid ethyl ester] are approved in Europe (Schnick 2001); benzocaine is also approved for use in Australia; isoeugenol is approved in Japan, Australia, New Zealand, South Korea, Costa Rica, and Chile; and eugenol is approved in Japan. Isoeugenol approval is pending in the United Kingdom and Norway.

Sedative Users

It is important to note that all of the aforementioned end users of fish sedatives are trained fisheries professionals. Demand for an immediate-release sedative is essentially restricted to a group of individuals with knowledge of fish biology and experience in handling fish. Thus, these individuals can be expected to demonstrate a significant level of knowledge when applying sedatives to fish. The likelihood of a novice user applying a sedative in an inappropriate manner—one that is inconsistent with the recommended use of the product or poses undue risk to themselves or the fish—is low.

Given the economic, social, and ecosystem values of fisheries and aquatic resources in North America and the importance of access to an immediate-release sedative for routine procedures and activities in the fisheries disciplines, it is essential to provide fisheries professionals and the scientific community with an immediate-release sedative to enable them to generate knowledge and manage resources effectively.

ASSESSING THE RISK ASSOCIATED WITH IMMEDIATE-RELEASE FISH SEDATIVES

Although it is difficult to quantify objectively, there is considerable risk associated with the continued absence of a suitable immediate-release sedative. This risk includes fish being handled without sedation, with CO_2, or with unapproved sedatives and the potential consequences of such actions. The consequences include morbidity and mortality of the fish, risk to the researchers and the environment, lost or flawed data, potential reliance on unapproved sedatives, and unwanted attention from the CVM's Surveillance and Compliance Team as well as the broader consequences of stocking failures, logistical challenges for domestic aquaculture, and management decisions based on inaccurate data. We contend that this level of risk is unacceptable.

Although it is difficult to quantitatively describe the risk associated with limited sedative options, it is easier to quantify the risk, or lack thereof, associated with using either benzocaine or eugenol-based compounds as immediate-release sedatives in the fisheries disciplines. The risk to humans of consuming benzocaine or eugenol is related to the probability that humans will consume treated fish, the residual concentration of the compounds in the fillets, and the human health consequences of the exposure. If food fish were treated during transport to market, immediate (within 1 h) consumption would represent a worst-case scenario for human exposure to benzocaine or eugenol via ingestion of treated fish. Other circumstances (e.g., the consumption of treated fish caught immediately upon release into the environment) are much less likely, given that treated fish may show temporary reluctance to feed following sedation. For example, fish collected by electrofishing (a standard fishery field collection technique) and sedated with either benzocaine or eugenol were less vulnerable to capture by angling than fish that had not been sedated (M. P. Gaikowski, USGS, unpublished data).

Risk can be described semiquantitatively by comparing the concentrations of sedatives known to cause adverse human health effects with the concentrations likely to be found in fish tissues given the worst-case scenario. For example, the exposure of Rainbow Trout and Largemouth Bass to a 50-mg/L benzocaine solution for 15 min resulted in muscle tissue levels varying from 10.6 to 14.0 mg/kg tissue (Guénette et al. 2007). Although a 15-min exposure exceeds that proposed for use of benzocaine for handling procedures, it can be considered a worst-case scenario. Assuming the maximum tissue accumulation levels observed by Guénette et al. (2007), a typical fillet portion (3 oz or 85 g) from a fish treated with benzocaine would contain an estimated 1.2 mg of benzocaine:

$$85\,\text{g fillet portion} \times \frac{1\,\text{kg}}{1{,}000\,\text{g}} \times \frac{14.0\,\text{mg benzocaine}}{\text{kg muscle tissue}} = 1.2\,\text{mg benzocaine per fillet portion.}$$

A survey of poison control centers (Suchard and Rudkin 2004) indicated that for acute exposures and ingestion rates greater than 5–40 mg benzocaine/kg body weight, observation or other interventions were recommended. Long-term exposure to benzocaine concentrations below this threshold does not appear to be problematic. Assuming the lowest ingestion tolerance (5 mg benzocaine · kg body weight^{-1} · d^{-1}) and the fillet portion concentrations calculated above (1.2 mg benzocaine/fillet portion), a 150-lb (68-kg) person could eat 283 portions of benzocaine-treated fish before ingesting the daily threshold concentrations associated with adverse effects:

$$68\,\text{kg person} \times \frac{5\,\text{mg benzocaine}}{\text{kg/day}} \times \frac{1\,\text{fillet portion}}{1.2\,\text{mg benzocaine}} = 283\,\text{portions per person per day.}$$

Even assuming a 10-fold margin of safety (i.e., assuming that individuals were to consume 10 times the number of portions they might actually consume or that portions were 10 times larger than is typical), consumers could still consume more than 9 treated fish portions at every meal each day without undue risk of health effects, if any serious risks exist:

$$\frac{283\,\text{portions}}{\text{person/day}} \times \frac{1\,\text{portion (actual)}}{10\,\text{portions (assumed)}} \times \frac{1\,\text{meal}}{1\,\text{portion}} \times \frac{1\,\text{day}}{3\,\text{meals}} = 9.4\,\text{portions per meal.}$$

Similar risk calculations can be made for eugenol-treated fish. Rainbow Trout exposed to a 75-mg/L eugenol solution for 15 min had maximal blood levels of 10.5 mg/kg (Guénette et al. 2007). The only muscle tissue data available for a clove derivative is for Rainbow Trout exposed to an 8.9-mg/L isoeugenol solution for 1 h, which yielded a maximal concentration of 55.4 mg isoeugenol/kg muscle tissue (Meinertz and Schreier 2009). Again, these exposures exceed the proposed durations for immediate-release sedative applications but can be used as worst-case scenarios. Assuming that eugenol accumulates in muscle tissue at a rate ranging from that associated with blood accumulation of eugenol to muscle accumulation of isoeugenol, a fillet portion from a fish treated with eugenol would contain an estimated 0.9–4.7 mg of eugenol:

$$85\,\text{g muscle tissue} \times \frac{1\,\text{kg}}{1{,}000\,\text{g}} \times \frac{10.5\,\text{to}\,55.4\,\text{mg eugenol}}{\text{kg muscle tissue}} = 0.9\,\text{to}\,4.7\,\text{mg eugenol per fillet portion.}$$

Clove and its derivatives, including eugenol, are generally recognized as safe and are found in many foods at concentrations exceeding 100 mg/kg. To avoid the long-term effects of eugenol exposure, the Joint FAO–WHO Expert Committees on Food Additives consider up to 2.5 mg · kg body weight^{-1} · d^{-1} an acceptable daily intake for humans (JECFA 2006). Assuming this ingestion tolerance and the highest fillet portion

concentrations calculated above (4.7 mg eugenol/fillet portion), a 150-lb (68-kg) person could eat 36 portions of eugenol-treated fish before exceeding the daily ingestion tolerance:

$$68\,\text{kg person} \times \frac{2.5\,\text{mg eugenol}}{\text{kg/day}} \times \frac{1\,\text{fillet portion}}{4.7\,\text{mg eugenol}} = 36\,\text{portions per person per day}.$$

Once again assuming a 10-fold margin of safety, consumers could still consume more than 1 treated fish portion at every meal each day without undue risk of health effects:

$$\frac{36\,\text{portions}}{\text{person/day}} \times \frac{1\,\text{person (actual)}}{10\,\text{portions (assumed)}} \times \frac{1\,\text{meal}}{1\,\text{portions}} \times \frac{1\,\text{day}}{3\,\text{meals}} = 1.2\,\text{portions per meal}.$$

Our approach to these risk calculations is highly conservative. We assumed that fish were consumed nearly immediately after treatment; that there was no metabolism or breakdown of the compounds in the body of the fish during storage or cooking of the fillets or in the body of the human consumer; that there was a 10-fold increase in consumption; and that there were no other risk-mitigating factors. Even so, this approach yields no evidence of increased risk of human health effects as a result of using benzocaine or eugenol-based compounds as immediate-release sedatives in the fisheries disciplines. The risk posed to humans resulting from the use of benzocaine or eugenol in this context is negligible and certainly less than the risk currently posed by the absence of an approved compound.

CONCLUSIONS AND RECOMMENDATIONS

We conclude that the absence of a suitable immediate-release sedative jeopardizes fish, fisheries, and fish culture and research and poses considerable risk to those involved in these activities and fisheries resources. The current candidate sedatives, benzocaine and eugenol, meet a range of criteria that justify an assumption of safety and efficacy as well as minimal risk to fish, researchers, the environment, and human consumers. The current framework and process for approving either of the candidate sedatives in the United States and other countries with similar regulatory arrangements will cost the private and public sectors an exorbitant amount of financial and human resources and will take years to complete. Risk management–based decision making does not appear to be consistently embraced by the agencies that regulate access to aquatic animal drugs. These agencies largely adhere to a process originally developed for the approval of human drugs and later modified for animals, with major species in mind (i.e., horses, dogs, cats, cattle, pigs, turkeys, and chickens). The CVM does consistently incorporate risk management–based decision making when evaluating NADAs for major species and has embraced the concept in some cases when evaluating NADA information for aquatic species. There are numerous examples of current CVM guidance that are, or appear to be, risk management based (USFDA 2001, 2003, 2006, 2009, 2011d), and the FDA fiscal year 2011 budget listed "improve[d] risk analysis and research for food and feed safety" as a priority (USFDA 2010). These examples signify a growing understanding of the value of risk assessment and risk management–based decision making and attempts to find innovative ways to meet legislative and regulatory requirements. Although there is a precedent for the use of risk management–based approaches to the safety and efficacy of human and animal drugs, these approaches remain underused in assessing drugs for aquatic species. As we have illustrated with the previous examples, the risk of the candidate immediate-release sedatives to humans is negligible; further, the risk to fish is minimal, given the nature of the drugs and the fact that end users are experienced fisheries professionals who are unlikely to make "first-time user" mistakes in applying the compounds. Although minor use/minor species legislation in the United States was intended to increase access to drugs for these applications, in practice the burden of evidence for an "all-fish approval" remains significantly greater than that for approvals for major species.

Accordingly, we recommend that risk management–based approaches—incorporating the characteristics and properties of fish drugs, the nature of their intended uses, and the experience of the prospective end users of these compounds—be implemented directly in the aquatic animal drug approval process. To this end, the AFS has adopted a policy statement, "Need for an Immediate-Release Anesthetic/Sedative for Use in the Fisheries Disciplines" (AFS 2011), which encourages the actions recommended herein and other specific measures to make the drug approval process more efficient in increasing the availability of safe and effective drugs, including immediate-release sedatives, in the fisheries disciplines.[4]

ACKNOWLEDGMENTS

The authors thank Christopher Guy for his contributions to the position paper from which the present manuscript was derived and John Bowzer for summarizing the attributes of currently available sedatives. We also thank the AFS Resource Policy Committee for their assistance in developing the concepts described herein as an AFS policy statement.

[4]During the final revisions of this manuscript, the FDA granted amended authorization for the use of AQUI-S 20E (10% eugenol) to allow for the immediate release of freshwater finfish sedated as part of field-based fisheries management activities. The amended authorization allows use of this product as described via the National Investigational New Animal Drug (INAD) Program under USFWS AADAP INAD 11-741. Although this temporary measure is not an approval and does not make the use of this product legal per se, it does provide a mechanism for accessing the product and offers fisheries professionals a means of addressing the concerns described herein.

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