Screening of Imported Tilapia Fillets for Heavy Metals and Veterinary Drug Residues in the Mid-Atlantic Region, USA

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Abstract

The objective of this study was to identify the presence of harmful drug residues (Chloramphenicol and malachite green/gentian violet) and toxic heavy metals (lead, cadmium, arsenic and mercury) in imported tilapia fillets. A total of 36 tilapia fillets were analyzed for these harmful chemical contaminants. The presence of veterinary drug residues were identified using competitive ELISA screening and the concentration of heavy metals were determined using a Perkin Elmer Graphite Furnace Atomic Absorption Spectrometer and Flow Injection Mercury System. Out of the 36 samples tested, none tested positive for chloramphenicol and malachite green / gentian violet. The fish samples, on average were found to have safe levels of mercury, cadmium, arsenic and lead in them, as set forth by the United States Food and Drug Administration (US FDA).

Keywords: Tilapia; Chloramphenicol; Malachite green; Arsenic; Lead; Cadmium; Mercury; Seafood safety

Practical Applications

The research with the tilapia samples provide a baseline for future studies related to seafood safety. This study will also help the regulatory agencies like the USDA and FDA in getting a better understanding of the prevalence of chemical residues in imported tilapia samples and henceforth be better able to focus on regulatory actions required.

Introduction

Tilapia has become one of the most popular seafood in the U.S. Consumption of tilapia has increased dramatically in the past decade. In 2002, tilapia was the 10th most popular seafood, with the per capita consumption being 0.143 Kg. However in 2010, tilapia was the 4th most consumed seafood with a per capita consumption of 0.657 Kg [1]. The import of tilapia from other countries has rocketed in recent years. In 2009, 183,294 MT of tilapia was imported into the U.S., valued at $696,086 million. In 2010, 215,378 MT of tilapia was imported which is valued at $842,866 million [2]. This is an astonishing 17 % increase in tilapia (in terms of metric tons) import as tonnage and 21 % increase in terms of dollar value.

In recent years, there have been several recalls of fish imported from overseas markets due to presence of harmful drug residues like melamine, chloramphenicol (CAP), malachite green (MG), gentian violet (GV) and heavy metals like lead (Pb), cadmium (Cd), chromium (Cr), iron (Fe), mercury (Hg), etc. Catfish from Indonesia, Thailand, Vietnam, Malaysia and other countries have been scrutinized for failure to comply with rules on antibiotic use [3]. The U.S. Food and Drug Administration recalled 927 tubs of Chinese crabmeat due to chloramphenicol contamination [4]. In June 2011, FDA recalled large quantities of crabmeat imported from Venezuela as the food tested positive for contamination [5].

Chloramphenicol (CAP) is known to cause bone marrow depression (IARC 1990) and has been banned by the FDA for use in food-producing animals [6]. Lu et al. [7] measured the concentration of CAP in three fish species sampled in China using liquid chromatography - tandem mass spectrometry and high performance liquid chromatography. The concentrations of CAP in the muscle tissues of carp, chub and grass carp were found to be 0.7, 0.35 and 0.24 g / kg, respectively [7].

Malachite green (MG) is a toxic chemical substance that is generally used to treat bacterial and fungal infections in fish [8]. MG is a mutagen and has been banned in the U.S. [9]. It is reported that MG is easily absorbed in fish tissue and can remain in the fish muscle in a reduced form (leucomalachite green) for a very long time [10]. MG has been reported to cause mutagenesis, carcinogenesis, chromosomal fractures, teratogenicity and respiratory toxicity [11]. Gentian violet (GV) has also been known to cause cancer and affect the urinary bladder, ovaries and uterus in mice [12]. In a study conducted by Food Standards Australia New Zealand [13], three Australian grown fishes and seven imported fish samples were found to have detectable levels of MG / LMG [13].

Humans can be exposed to toxic heavy metals through various sources. Water pipes, paint and gasoline are common sources of Pb exposure. At low concentrations Pb can affect learning and memory and at higher doses it can cause poisoning [14]. Amongst all aquatic species, fish are most susceptible to heavy metal contamination [15]. Humans are usually exposed to methyl mercury (Hg) through consumption of fish such as shark, swordfish and tuna. There is also bioaccumulation of Hg in fresh water fish that live in contaminated lakes. Consumption of contaminated fish can pass the mercury to humans. Mercury at high doses can cause cerebral-palsy in children. Arsenic (As) is a known carcinogen for humans and cadmium can affect the male reproductive systems. Dental amalgam filling is one of the main routes for inorganic mercury contamination. Cadmium (Cd) exposure can come from semi-conductor manufacture, metal plating, ceramic plating, shellfish and contaminated water [14].

The concentration of different heavy metals such as cobalt (Co), cadmium (Cd), lead (Pb), iron (Fe) and copper (Cu) were determined

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in six different species of fishes found along the Libyan coastline. The concentrations of heavy metals were determined using flame atomic absorption spectrometry. The study showed that the accumulation of Co, Cd, Pb were more than that of the Cu accumulation in all six species of fish [16].

Mol [17] measured the concentration of trace metals (Fe, Zn, Cu, Cd, Sn, Hg and Pb) in canned rainbow trout and canned anchovies. The mean concentrations of trace metals in canned anchovies were 50.708 mg/kg for Fe, 22.467 mg/kg for Zn, 1.145 mg/kg for Cu, 0.019 mg/kg for Cd, 0.140 mg/kg for tin, 0.041 mg/kg for Hg and 0.188 mg/kg for Pb. The mean concentrations of trace metals in canned rainbow trout were as follows: 6.980 mg/kg for Fe, 11.605 mg/kg for Zn, 0.541 mg/kg for Cu, 0.001 mg/kg for Cd, 0.023 mg/kg for Sn, 0.026 mg/kg for Hg and 0.167 mg/kg for Pb. The study showed that the Fe and Pb content in some of the samples were above the permissible limits [17]. The bioaccumulation of different heavy metals in Tilapia nicolitica sp. found in rivers of Bayelsa state (Nigeria) was also studied. The mean concentration of Cd in the flesh was 0.95 ± 0.20 mg / kg. The concentrations of Co, Ni and Pb were measured to be 6.45 ± 2.45, 4.48 ± 1.47 and 3.88 ± 0.02 mg / kg, respectively [18]. Oates and Stucky [19] reported Cd levels ranging from 0.18 ppm to 0.44 ppm in catfish collected from Nebraska waters. The Pb concentration in samples from the same study ranged from 0.05 ppm to 0.75 ppm. One of the catfish contained 0.85 ppm Hg [19].

Our primary objective in this study was to sample imported tilapia fillets from retail establishments in Delaware, Maryland, the District of Columbia and Virginia to provide baseline data on the incidence and prevalence of heavy metal contamination including As, Pb, Hg, Cd and the drug residues; MG, GV, and CAP.

Materials and Methods

The following equipment and chemicals/reagents were used in the research study conducted to identify fish samples with contaminants.

Instrumentation

The concentration of As, Pb and Cd was measured using a Perkin Elmer Atomic Absorption Spectrometer (Analyst 600). A Perkin Elmer Flow Injection Mercury System (FIMS 100) was used to measure the concentration of Hg in tilapia samples. The absorbance values obtained in drug residue screening experiments were measured using a BioTek ELISA reader (BioTek Instruments, Winooski, VT).

Chemicals and reagents

The following commercial ELISA kits were used for screening fish samples for the presence of veterinary drug residues. BioControl TRANSIA Plate Chloramphenicol Test Kit (BioControl, Bellevue, WA) was used for Chloramphenicol screening and BioScientific Malachite Green / LMG ELISA Test Kit (Bio Scientific, Austin, TX) were used for MG / GV screening. The stock calibration standards (1000 ppm of Hg, Pb, Cd and As) for heavy metal testing were purchased from SCP Science (SCP Science, Champlain, NY). The chemicals used for drug and heavy metal testing like the n-hexane, acetonitrile, dichloromethane, ethyl acetate, HPLC grade water and acids were purchased from Fisher Scientific (Fisher, Savannah, GA).

Sample collection and preparation

The tilapia samples were purchased from retail outlets in Maryland, Delaware, Virginia and the District of Columbia on Sundays. A minimum of three tilapia fillets were purchased for each fish sample. Care was taken to avoid purchasing fillets that were not properly identified as tilapia. Also, the fish samples were purchased on the pretext of being a regular customer (a secret shopper). This was done to make sure the tilapia samples purchased represent the real world scenario of purchasing fish in a supermarket. The details of the fish purchase (such as storage conditions, country of origin, store address, handling techniques and any oddities) were noted down on a sample information sheet furnished by the USDA-FSIS. A total of 108 tilapia fillets, each tilapia sample was made from three individual tilapia fillets (subsamples) resulting in 36 tilapia samples, were purchased from 4 different states over a period of 8 weeks. No two tilapia samples were purchased from the same retail store. Also, the number of stores that had imported tilapia fillets were much less than we initially planned in the DELMARVA region limiting the total sample number to 36. The fish samples were then transported to the lab in an ice cooler kept at about 4 °C.
4°C. The fish samples were then blended within 24 hr using a generic blender (Kitchen aid, New York, USA) to yield a fine paste of tilapia sample. Contamination of samples by external agents was avoided by thoroughly cleaning the blender and all surfaces using deionized water. The blended fish samples were labeled and stored at -20°C until they were tested for contaminants. The process flow for sample collection and preparation is shown in Figure 1A. Specifically, Figure 1B depicts the tilapia sample preparation step.

Control tilapia was collected from the Delaware State University aquaculture ponds. The control fish tissue was confirmed to have no detectable levels of mercury, lead, cadmium or arsenic. They were also found to be free of drug residues: CAP and MG/GV. Figure 2 shows the sample distribution based on the country of origin.

Screening for veterinary drug residues

Chloramphenicol screening: The presence of CAP in tilapia samples was identified using competitive ELISA-based screening. The proficiency / minimum level detection for chloramphenicol testing was 0.25 ppb [9]. All the below experimental steps were adapted from the USDA-FSIS, CLG-CAM1.01 “Screening for Chloramphenicol” protocol. Solvent extraction techniques were employed to extract any chloramphenicol present in the fish samples and screened using the ELISA test kit from BioControl.

The analysis was performed in triplicates. Initially, 3 ± 0.1 g of the blended tilapia samples were weighed in 50 ml propylene centrifuge tubes. The experiment also involved recovery, negative and positive control samples. Positive and recovery controls were made by fortifying the control tilapia with known concentrations of CAP solution (from the ELISA kit). To each sample tube, 6 ml of ethyl acetate was added and shaken vigorously in a mechanical shaker for 10 min. The samples were then centrifuged at 716 x g for about 10 min and 4 ml of the supernatants were transferred to 15 ml glass centrifuge tubes. The supernatant was then evaporated in the nitrogen evaporator until all supernatant was fully evaporated (approximately 30 min). To each glass centrifuge tube, 1 ml of n-hexane and 1 ml of 1X sample dilution buffer was added. The tubes were then vortexed for 1 min and centrifuged at 716 x g for 10 min. The lower aqueous phase was used for the ELISA screening.

The screening consisted of six ELISA wells for positive controls, 2 wells for recovery, 4 wells for negative controls, standards (0.05, 0.1, 0.2, 0.5 and 2 ppb) and samples. To the first two wells, 100 μL of the reconstitution buffer was added to serve as a plate blank. To the next two wells, 50 μL of the same reconstitution buffer was added to serve as zero standards. To different wells, 50 μL of the standards, positive controls, negative controls and samples were added. To each well (except the plate blank wells), 25 μL of reconstituted conjugate solution was added, followed by 25 μL of the reconstituted antibody solution. The ELISA plate was shaken for 1 min and then incubated at about 5°C for 1 hr.

The wells were then washed three times using 300 μL of the 1X washing buffer. The washing buffer was then allowed to drain and any bubbles that were present in the wells were eliminated under a flowing nitrogen gas stream. To each well, 100 μL of the substrate solution was added and incubated for 30 min at room temperature. The last step was to add 100 μL of the stop solution to all wells and the absorbance of the ELISA plate was read at 450 nm using the BioTek reader (Figure 3). The variability in absorbance had to be ≤25 % for the test set to be considered acceptable. The standard deviation (SD) and mean for the absorbance values of positive controls (six wells) were calculated. The decision level (DL) for the experimental set was calculated using the formula ‘DL = average + (3*SD)’. A tilapia sample that had the mean absorbance value less than or equal to the decision level (DL) was considered a presumptive positive.

Malachite green/gentian violet screening: The ELISA screening cannot differentiate between Leucomalachite Green (LMG), Malachite Green, Leucocrystat Violet and Crystal Violet [20]. The quantitative analysis is only capable of giving results that are a total sum of concentrations of these four compounds. This ELISA analysis is capable of detecting the total Crystal Violet, Leucocrystat Violet, Malachite Green and Leucomalachite Green at concentrations more than 1 ppb [20]. In order to minimize the costs of sampling and best use of the resource that kindly availed by the USDA-ARS ERRC Residue Chemistry and Predictive Microbiology Research Unit, only samples tested presumptive positive with the ELISA Screening were tested using HPLC-tandem mass spectrometry. Although HPLC is a sensitive instrument to detect drug residues, it is expensive to operate the instrument. ELISA screening is a cost and time effective tool to weed out presumptive negatives, leaving us with very small percent of presumptive positive samples. These presumptive positives can later be analyzed with HPLC as a confirmatory analysis. Also, retail stores and seafood distributors can randomly screen for veterinary drug residues using ELISA screening if they choose to do so with minimal training. However, it would be very costly and time-consuming for the distributors and store owners to utilize HPLC as a screening tool for drug residues.

The ELISA analyses were performed in triplicates. For each analysis, 2 ± 0.1 g of the homogenized tilapia samples were weighed in...
50 ml polypropylene centrifuge tubes. The analysis set also consisted of a control tilapia samples. The positive controls were fortified with 50 µL of the MG spiking solution. The negative controls were spiked with 50 µL of negative control solution from the ELISA kit. To each centrifuge tube, 0.6 ml of 1 X sample extraction buffer A, 0.4 ml of sample extraction buffer B and 6.5 ml of acetonitrile solutions were added and vortexed for 3 min. Then, 3 ml of HPLC water and 2 ml of dichloromethane were added to each tube. After vortexing for 1 min, the tubes were centrifuged for 5 min at 716 x g. Three ml of the upper organic layer was then transferred to 15 ml centrifuge tubes containing 100 µL of 1 X oxidant solution. The sample was incubated at room temperature for about 25 min to allow oxidation of LMG and LCV to MG and CV, respectively. Then each centrifuge tube was placed in the nitrogen evaporator kept at about 60°C, where the samples were brought to complete dryness by blowing nitrogen gas into the sample tubes. Then, 2 ml of n-hexane and 1.6 ml of buffer D/acetonitrile solutions were added to the centrifuge tube. The tube was mixed well and centrifuged for 10 min at 716 x g.

The ELISA plate was inoculated with samples and standards in triplicates, 6 wells for positive controls, 4 wells for negative controls and 2 wells for the recovery. To the ELISA wells, 90 µL of the sample from the lower organic phase was added. Then, 30 µL of the biotin conjugate was added and incubated for 30 min at room temperature. Following this, the wells were washed three times with 250 µL of 1X wash buffer. After that, 100 µL of streptavidin conjugate was added to all wells and incubated for 15 min at room temperature. This was followed by another set of 3 washing steps with 1X washing buffer. To each well, 100 µL of the substrate solution was added and left for incubation at room temperature for 15 min. Finally, 100 µL of the stop solution was added to all ELISA wells to stop the enzymatic reaction (Figure 4). The absorbance value of the color developed was measured at 450 nm using the BioTek ELISA reader. The presumptive positive samples were identified using the same decision level formula illustrated in the previous section.

**HPLC-MS/MS confirmation for veterinary drug residues:** The tilapia samples that tested presumptive positive for veterinary drug residues was confirmed by HPLC-tandem mass spectrometry following a standard European Union (EU) method originally developed in Fougères Laboratory, the French Agency for Food, Environment and Occupational Health Safety (ANSES) [21]. Deuterated internal standards were used for both confirmation and quantification. The Liquid Chromatography analysis was done using an Agilent 1100 liquid chromatograph system. A Prodigy ODS-3 (3 µm, 100 mm x 2.0 mm) C18 column (Phenomenex, Torrance, CA) was used at 30°C with a C18 Security Guard ODS (4.0 x 2.0 mm) guard column (Phenomenex, Torrance, CA). The detector was an Applied Biosystems/MDS SCIEX API 3000TM triple-quadrupole mass spectrometer (Concord, Ontario, Canada) fitted with an electrospray ionization (ESI) source and monitored by Analyst 1.5 software.

**Heavy metal analysis**

**Arsenic (As) analysis:** The arsenic concentration in tilapia samples was determined following the USDA-FSIS CLG-ARS1.04 protocol [22]. The homogenized fish samples were weighed (5 ± 0.1 g) in 50 ml Vycor crucibles (Corning, Lowell, MA). Each analysis set contained samples, control, fortified control and reagent blank. Then, 4 ml of 50% Mg(NO₃)₂, was added to each sample and mixed well. The samples were then dried and ashed in a Thermolyne Muffle Furnace (Thermo Fisher Scientific, Waltham, MA). The ashing steps involved three levels of heating for different periods of time. In the first step, the samples were dried at 100°C for 360 min, followed by heating at 150°C for 360 min and then finally ashed at 500°C for about 480 min. The samples were allowed to cool at room temperature. Then, 3 ml of 50% HNO₃ was added to all crucibles. The samples were then heated on a hot plate to dry the ash, followed by heating at 550°C for 60 min. Upon cooling at room temperature, the following three solutions were added; 10 ml of 4.5 N HCl, 35 ml of 10% HCl and 5 ml of 10% potassium iodide / ascorbic acid solution. The concentration of arsenic in acid solution was measured using the Atomic Absorption Spectrometer (AAS 600). The matrix modifier used in arsenic analysis was 0.1 % palladium with 0.06 % Mg(NO₃)₂. A mixture of 15 µL of matrix modifier and 20 µL of the sample solution was injected into the AAS 600 (by auto sampler) for arsenic analysis. The sample solution was analyzed in triplicates using the AAS 600. The method was validated by spiking the control tilapia sample with known concentrations of arsenic standards and the analysis was done in triplicate to validate the analytical method.

**Lead (Pb) and cadmium (Cd) analysis:** The lead and cadmium analysis was performed according to USDA-FSIS CLG-TM1.01 protocol [23]. For lead and cadmium analysis, 15 ± 0.1 g of the finely blended tilapia sample was weighed in a 50 ml Vycor crucible. Then, 7.5 ml of 6.67 % Mg(NO₃)₂, was added to each sample and mixed well. Each sample was heated in the muffle furnace for about 360 min at 95°C. The second step was to ash the samples in the furnace at 550°C for 960 min. The samples were taken out of the furnace and allowed to cool to room temperature. In the second ashing step, 2 ml of 50% HNO₃ was added to each sample and heated at 550°C for 60 min. The last step was to dissolve the ash in 15 ml of 1.0 N HCl, transfer 15 ml to polypropylene centrifuge tubes, and store until further analysis using the AAS 600. The matrix modifier used for lead analysis was a mixture of 1% ammonium phosphate and 0.06 % Mg(NO₃)₂. For cadmium analysis, a solution of 1 % palladium and 0.06 % Mg(NO₃)₂, was used as the matrix modifier. The sample solution was analyzed in triplicates using the AAS 600. Spiked control samples were used in every sample set to validate the results obtained from lead and cadmium analysis.

**Mercury (Hg) analysis:** Mercury analysis was done using the USDA-FSIS CLG-MERC1.00 protocol [24]. For mercury analysis, 0.3 ± 0.1 g of the tilapia samples was weighed in 300 ml Biochemical Oxygen Demand (BOD) bottles. To each bottle, 1 ml of 70% HNO₃ and 4 ml of H₂SO₄ was added and mixed well. The BOD bottles were then incubated in a water bath set at 80 ± 5°C for 30 min. The bottles were later taken from the water bath and allowed to cool. To the BOD bottles, the following two solutions were added; 15 ml of K₂MnO₄ and 8 ml of 5% K₂S₂O₇. The BOD bottles were then incubated in a water bath for 90 min at 30 ± 5°C. Then, 10 ml of the solution from the BOD bottles were transferred to 15 ml polypropylene centrifuge tubes. To each centrifuge tube, 750 µL of 12% hydroxylamine solution was added. The solution was then tested for mercury concentration in triplicates.
using the Perkin Elmer Flow Injection Mercury System (FIMS 100). Method validation was done by spiking the control tilapia sample with mercury standards and measuring the known concentration.

Results and Discussion

The ELISA screening for veterinary drug residues identified three tilapia samples that were presumptive positive for Malachite Green / Gentian Violet. Of the three samples, two fish samples originated from Ecuador and one sample was from Honduras; however, no sample tested presumptive positive for chloramphenicol. Three samples that tested presumptive positive for MG / GV with ELISA Screening were later sent to the USDA-ARS Eastern Regional Research Center (ERRC) for confirmatory test using the HPLC-MS/MS. No tilapia sample was found to have any violative levels (more than 1 ppb) of MG and GV in the confirmatory tests. Previous research conducted by the USDA-FSIS [20] reported that about 1% of the catfish samples tested were confirmed to have trace levels of Malachite Green. Also, the same study concluded that none of the imported samples had any Chloramphenicol USDA-FSIS [20].

David et al. [25] gathered the drug residue inspection data from four major countries / inspection bodies, namely The European Union, United States, Canada and Japan. Out of the total drug residue violations reported from all four inspection agencies, tilapia accounted for 0-5 % of the total safety violations from 2001-2009. Out of the 138 drug residue related violations in the USA between 2001-2006, less than 2% were for tilapia. However, tilapia related violations accounted for close to 5% of the total violations in the European Union and Canada. Almost 43% and 23% of the drug tainted seafood imported into the USA from 2001-2006 originated from Vietnam and China, respectively [25]. Of the samples that we tested, none originated from Vietnam and less than 20% of our samples originated from China. This is possibly why none of the tilapia samples that we tested was positive for the veterinary drug residues.

Nearly 70% of the tilapia samples that we tested originated from Ecuador, Honduras and Costa Rica (Figure 2) possibly because most retailers in the four states sampled only had tilapia fillets imported from Ecuador. This could possibly be due to the fact that these countries are in closer proximity to the east coast of the U.S. We may have been able to identify tilapia samples for drug residue violations if we were able to collect tilapia samples that originated from Vietnam. Table 1 shows the results of our veterinary drug residue testing using the ELISA screening and HPLC-MS/MS.

| Origin of Sample | Number of Samples Analyzed | Percent Sampled (%) | Samples tested presumptive positive for CAP using ELISA | *Samples tested presumptive positive for MG/GV using ELISA |
|------------------|---------------------------|---------------------|-------------------------------------------------|-------------------------------------------------|
| Ecuador          | 19                        | 52.77               | 0                                               | 2                                               |
| Honduras         | 5                         | 13.88               | 0                                               | 1                                               |
| China            | 7                         | 19.44               | 0                                               | 0                                               |
| Indonesia        | 2                         | 5.55                | 0                                               | 0                                               |
| Costa Rica       | 1                         | 2.77                | 0                                               | 0                                               |
| India            | 1                         | 2.77                | 0                                               | 0                                               |
| Colombia         | 1                         | 2.77                | 0                                               | 0                                               |
| Total            | 36                        | 100                 | 0                                               | 3                                               |

*Confirmatory tests for MG/GV were done using HPLC-MS/MS at the USDA-ARS ERRC for the samples which were presumptive positive with ELISA Screening. The confirmatory results were negative for MG/GV for all three samples.

Table 1: Results of veterinary drug testing through competitive ELISA screening and HPLC-MS/MS.

Figure 5: Mean concentration (± standard error bar) of each tilapia sample (sample=3 fillets) analyzed for a) arsenic (as), b) lead(gb), c) cadmium (cd), and d) mercury (hg).
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| Heavy metal | Range (ppb) | Mean concentration in tilapia samples (ppb) | #Action Level in Seafood (ppb)¹ | Mean concentration of heavy metal consumed in ppb / Kg /day | EPA Chronic Oral RfD |
|------------|-------------|--------------------------------------------|-------------------------------|--------------------------------------------------------|----------------------|
| Arsenic    | 0-55.85     | 2.88                                       | 76,000-86,000                 | 3.0                                                    | None set             |
| Lead       | 0-4.5       | 1.16                                       | 1500-1700                     | 10.0                                                   | None set             |
| Cadmium    | 0-85.28     | 10.89                                      | 3000-4000                     | 10.0                                                   | None set             |
| Mercury    | 0-156.8     | 17.48                                      | 1000                          | 1.0                                                    | None set             |

¹Levels set by US FDA, it is worthwhile noting that the EPA Chronic oral exposure (RfD) levels are more suggestive of the health hazards of consuming food tainted with heavy metals (Table 3).

Burgera and Gochfeld calculated the safeness of seafood by determining the risk involved with consuming 8-ounces (228 g) seafood/meal/week. The study also assumed that an average person would weigh 70 Kg. Based on that assumption, we calculated the amount of these toxic heavy metals a person would intake if they consumed the tilapia that was tested in this study. It is alarming to notice that the mercury consumed in one meal (8.13 ppb/day) would be nearly eight-fold more than what EPA has outlined for oral intake/day (1 ppb/day) [26]. The levels of arsenic and cadmium in the tilapia samples were found to be less than the RfD levels set forth by EPA. EPA does not have a chronic oral exposure (RfD) level for lead. The RfD values for different heavy metals is shown in Table 3.

Table 2. Range, mean concentration and action levels for heavy metal concentrations in seafood.

Table 3. Mean concentration, concentrations of heavy metal consumed in one day, and EPA chronic oral RfD.

levels set by US FDA, it is worthwhile noting that the EPA Chronic oral exposure (RfD) levels are more suggestive of the health hazards of consuming food tainted with heavy metals (Table 3).

Conclusion

Our research focus in this project was on the safety consideration of the tilapia fillets for consumption by local citizens purchasing them at the local seafood and supermarkets and is believed to serve as good baseline information for the public and federal agencies which monitor and regulate the imported seafood. This research study clearly demonstrates the need to thoroughly monitor the quality of seafood imported into the U.S. Currently, less than 2% of the imported seafood is tested for FDA and microbial contaminants. From an economic standpoint, testing 100% of imported seafood is not feasible. However, FDA must increase the percent of samples tested to ensure the safety of the American public. Also, FDA must encourage the retailers to sell U.S. raised tilapia fillets alongside imported seafood, because it is almost impossible to find domestic tilapia fillets at retail outlets visited during the study. The USDA-FSIS has a special wing called the "Office of Catfish Inspection" to monitor the quality of domestic and imported catfish. It would be beneficial to have a similar activity to monitor the quality of imported tilapia to ensure that consumers have healthy and safe choices in seafood.

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