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Assessment of Antibiotic Residues, Pesticides and Malachite green in Local Fish

Ph.D. Thesis

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List of Abbreviations

Abbreviation	Word
ADI	Acceptable daily intake
AOAC	Association of Official Analytical Chemists
ATSDR	Agency of Toxic Substances & Disease Registry
δ-BHC	Delta-benzene hexachloride
CR	Carcinogenic risk
CSF	Cancer slope factor
DDD	dichlorodiphenyldichloroethane
DDE	chlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
EC	European Commission
EDI	Estimated daily intake
FDA	Food and Drug Administration
FSIS	Food Safety and Inspection Service
HCH	hexachlorocyclohexanes
HPLC	High-performance liquid chromatography
HR	Human risk
LCMs	Liquid chromatography–mass spectrometry
MG	Malachite green
MRL	Maximum residual limit
OCPs	Organochlorine pesticides
OTC	Oxytetracycline

removed using a stainless-steel knife. The soft parts of fish samples were removed and a muscle tissue sample (50g) was taken from the dorsal muscle and prepared for pesticides extraction at the same day of collection.

3.1.3.1.2 Extraction of OCPs residues:

Fifty grams of fish samples were thoroughly ground into high-speed blender, 100 g anhydrous sodium sulphate were added to combine with water present and disintegrate the test portion. Alternately blended and mixed with spatula until sample and anhydrous sodium were well mixed. Scrape down sides of blender jar and break up cake material with spatula, we added 150 ml petroleum ether and blended at high speed for 2 minutes.

The extract then decanted through 12 cm Buchner funnel (containing two 12 cm Whatman filter paper No. 1) into a suction flask. The residue in the blender cup was re-extracted with 75 ml petroleum ether, blended at high speed for 2 minutes decanted through the Buchner funnel and collected with the first extract. The obtained extract was put in rotary evaporator until complete evaporation of petroleum ether and obtaining only fat content.

3.1.3.1.3Clean-up by Petroleum ether-Acetonitrile partitioning:

The extracted fat was transferred into 125 ml separator funnel and 15 ml petroleum ether and 30 ml acetonitrile saturated with petroleum ether were added. We shacked the funnel vigorously for 1 minute and hold the separator funnel in horizontal position and allow the layers to separate then drain the acetonitrile layer (the lower one) into 1 L another separator funnel containing 650 ml distilled water. We mixed 100 ml petroleum ether and 40 ml saturated NaCl solution and for 1 minute and leaved to separate then the ether layer (the upper one) drained over cotton wool and anhydrous sodium sulfate conditioned with petroleum ether into 250 ml round bottom flask. Then we evaporated it till complete evaporation of petroleum ether.

3.1.3.1.4 Florisil Column Clean-up:

A glass column 22 mm internal diameter was blocked from bottom with glass wool and filled with florisil (60-100 mesh pesticides residues grade activated at 130 ºC for 12 hours) to a height of 10 cm topped with 1 cm anhydrous sodium sulfate. Pre-wet the column with 40-50 ml petroleum ether then add 2g anhydrous sodium sulfate and 20 ml petroleum ether to the obtained petroleum ether solution of sample extract from the above step then passed through the prepared column at the rate of 5ml/minute.

The column was eluted at the same rate (5ml/minute) using 20 ml from every one of three eluting solvents (6% diethyl ether in petroleum ether, 15% diethyl ether in petroleum ether and 50% diethyl ether in petroleum ether). The eluate was concentrated to a dry film using the rotary evaporator then dissolved in 2ml n-hexane and transferred into autosampler vial for GC-analysis.

3.1.3.1.5 Quantitative determination of organochlorine pesticides:

The extracts were injected into gas chromatography apparatus **(Agilent GC model 6890)** equipped with an Ni^{63} electron capture detector (ECD), capillary column of 30 m length, 0.32mm internal diameter, and 0.25 µm film thickness. The oven temperature was programmed from an initial temperature 160 ºC (2 min hold) to 280 ºC at a rate of 5º C /min and maintained at 280ºC for 10 min. Injector and detector temperatures were maintained at 280 and 320 ºC, respectively. Nitrogen was used as a carrier gas at flow rate of 4 ml /min and injection volume of 1µl.

The pesticide residues were identified based on comparison of relative retention times to those of known standards, stock standard solutions of pesticide were prepared by dissolving the compound in hexane and stored in amber bottles.

7-Summary

Sixty local fish samples (each sample represented by one kilogram) were randomly collected from various regions at Damietta governorate (Egypt).

The first part was a survey where 20 from each mullet and tilapia, and 10 from each bass and shrimp samples analyzed for their contents of antibiotics, organochlorine pesticides, and malachite green residues. The results revealed that antibiotics residues were not detected in the examined fish samples.

While for OCPs residues, the mean ±S.E values for DDT in mullet, tilapia, bass, and shrimp were 5.1± 0.64, 18.3±2.54, 3.17± 0.73, and 9.75± 0.52 ppb, respectively. For DDD were 52.7±0.97, 80.5± 8.61, 4.17±0.44, and 29.63±0.55 ppb, consecutively. However, DDE residues in mullet, tilapia, and shrimp were 7.56±0.75, 46.6±9.77, and 13.3±0.32 ppb. Alderin residues in mullet and tilapia were 89± 0.85, 40.3±3.72 ppb. Dieldrin mean values in mullet and tilapia were 36.7± 0.66, and 35.75±5.51ppb. However, Heptachlor residues in mullet, tilapia, and shrimp were 20±0.59, 8.38±1.62, and 17.2±0.56 ppb. Heptachlor epoxide residues in mullet, tilapia, and shrimp were 38.9±0.49, 13.1±2.38, and 30.4±0.57 ppb. While for α HCH residues in mullet and tilapia were 15.3±2.44 and 48±10.75 ppb, while γ –HCH, endosulfan and γ-chlordane residues in tilapia were 2.8±0.44, 12.8±1.21, and 79.5±17.37 ppb, respectively, that not detected in other examined fish.

The mean ± S.D values of malachite green residues contents in mullet, tilapia, bass, and shrimp were 1.558 ± 0.165, 1.374± 0.326, 0.719 ± 0.148, 1.213± 0.130 ppb, respectively. While the minimum values were

< 0.3 ppb and the maximum residues levels were as 2.61, 2.76, 1.18, 1.43 ppb respectively. The levels that was more than the maximum residue limit (2 ppb) in all examined fish samples was as 6 (10%).

The second part was a control part, which aimed to study the effect of heat treatment (microwaving, roasting and boiling) for DDD residues, showed the reduction percentage of microwaving, roasting, and boiling was 81.13%, 60.38%, and 79.25%. For DDE residues, the reduction percentage microwaving, roasting, and boiling was 100%, 7.14%, and 71.43%. DDT residues were completely reduced in all cooking methods, the reduction percentage of heptachlor residues by roasting was 86.36%, and completely reduced by microwaving and boiling. The reduction percentage of heptachlor epoxide by microwaving and roasting was 94.88%, and 79.49%, and completely reduced by boiling. α- HCH residues were reduced by 95.83% for roasting and completely reduced by microwaving and boiling techniques. While γ-HCH was reduced 100% in all cooking methods. Alderin residues were reduced in microwaving, roasting, and boiling by 82.02%, 67.42%, and100%. Dieldrin residues were completely reduced in all cooking methods (100%). Endosulfan and γ-chlordane residues were reduced by 82.02%, 67.42 %, and 100% in microwaving, roasting, and boiling, respectively.

Mean values and the reduction percentages (%) of malachite green residues were 0.24±0.13 ppb (81.80%), and 0.88±0.50 ppb (32.90%) after microwaving and roasting of analyzed fish samples. Malachite green residues were completely reduced (100%) by boiling method in all examined fish samples.