DETECTION AND MINIMIZATION OF HIGH RISK PERSISTENT CHEMICAL POLLUTANTS IN RATIONS, MILK AND THEIR PRODUCTS

By

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B.Sc. Agric. Sciences (General Branch), Cairo University, 1999
MSc. Environmental Agric. Sciences, Ain Shams University, 2009

THESIS
**Submitted In Partial Fulfillment Of
The Requirement for the Degree of**

DOCTOR OF PHILOSOPHY
**In
Environmental Sciences**

**Department of Environmental Agricultural Sciences
Institute of Environmental Studies and Research
Ain Shams University
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<u>APPROVAL SHEET</u>

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ABSTRACT

DETECTION AND MINIMIZATION OF HIGH RISK PERSISTENT CHEMICAL POLLUTANTS IN RATIONS, MILK AND THEIR PRODUCTS

In the present study, (59) animal feed and (35) cow milk samples had been collected and analyzed to detect the levels of contamination by persistent organic pollutants (dioxins and dl-PCBs); firstly at certain Egyptian farms which represent the eastern, western and middle Egypt including the following governorates: Qaliubiya, Sharkia, Monoufia, Damietta, Kafr El-Sheikh, Gharbiya, Ismailia, Port Said, Noubaria (Beheira), Fayoum and Beni Sweaf; secondly as continuous, (92) animal feed and (106) different varieties of processed cow liquid milk samples; including full cream milk (69), half cream milk (22) and skimmed milk (15) had been collected and analyzed for these pollutants from the big markets in terms of geographical distribution in which represents the same location farms. The obtained results indicated that the highest (TEQ) concentration of Σ(PCDD/Fs + dl-PCBs) was founded in Kafr El-Zayat farm (Gharbiya governorate) which represent rural areas and nearby industrial zones; hence the cow milk, animal feed (vegetable oils) and clover (green fodder) samples were up to 14.60 pgTEQ.g^{-1} fat weight (fw), 1.81 and 1.48 pgTEQ.g^{-1} dry weight (dw), respectively in which exceed the EU maximum limits in food of 6 pg WHO-TEQ g^{-1} fat weight and the animal feed samples were exceed the EU Maximum limits of 1.25 pgWHO-TEQg^{-1} dry weight. However, the lowest (TEQ) concentration of Σ(PCDD/Fs + dl-PCBs) was founded in El-Noubaria farm (Beheira governorate), sited in the modern reclaimed lands near urban areas; hence the cow milk was 1.48 pg TEQ g^{-1} fw and the animal

feed sample as [soybean-cereals] was 0.09 pg TEQ g^{-1} dw, there were below the EU limits, respectively. Other samples collected from markets, was observed that, the dioxins contaminated samples which exceeded the European maximum permissible levels **(EC, 2006)** were up to (37.7 and 9%) in processed milk and animal feed, respectively. While for dl-PCBs, there were exceeded the EU limits with ratio (1 and 0%) in processed milk and animal feed, respectively. Regarding to ADI, our results proved that the mean current dietary intake from consumption of milk for PCDDs/PCDFs, dl-PCBs and total TEQ which represent 0.35 pg WHO-TEQ kg^{-1} body weight day^{-1} with ratio (8.6%) was below the international acceptable daily intake (ADI) which is 1 – 4 pg WHO-TEQ kg^{-1} bw day^{-1}. To mitigate the PCDD/Fs and dl-PCBs in the contaminated full cream and skim milk samples, Laccase enzyme was added in different concentrations (3.6, 7.2, and 10.8 unit) under contact time of 2hr at 30°C. In general, the degradation of PCDD/Fs and dl-PCBs were markedly observed in full cream milk samples, where the highest degradation were obtained using 10.8U laccase in which a total of 21 and 23% reduction for PCDD/Fs and dl-PCBs, respectively. On the other hand, very limited reduction was occurred in skim milk samples, even when using higher laccase conc. of 10.8U, whereas reduced as a total of 6 and 14% for PCDD/Fs and dl-PCBs, respectively. After measurements of fat contents, protein and pH; it was observed that, there were no effects obtained on the fat, total protein contents and pH of milk samples. The highest degradation efficiency of dioxin and dl-PCBs congeners, was observed for 1,2,3,7,8,9-HxCDD of 28 and 8%, and PCB118 of 49 and 19% in full cream milk and skim milk, respectively. Whereas, the lowest degradation efficiency of dioxin and dl-PCBs congeners, was obtained for 2,3,7,8-TCDF of 16 and 6%, and PCB81 of 12 and 8% in full cream

milk and skim milk, respectively. Thus, it can recommend to use laccase enzyme to mitigate the persistent organic pollutants (dioxins and dl-PCBs) from contaminated feed and milk products to produce safe food products.

LIST OF CONTENTS

LIST OF CONTENTS

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviate	Description in details
ACF	Activated carbon fiber
ADI	Acceptable daily intake
AhR	Aryl hydrocarbon receptor
ASE	Accelerated Solvent Extraction
APGC–MS/MS	Atmospheric Pressure Gas Chromatography–Tandem Mass Spectrometry
CRMs	Certified reference materials
COE	Carry over effects
CORs	Carry-over rates
CC-SCE	Counter current supercritical CO_2 extraction
DRCALUX	Dioxin-responsive chemically activated luciferase gene expression
DL-PCBs	Dioxin-like polychlorinated biphenyls
EDI	Estimated daily intake
EMI	Estimated monthly intake
EHDI	Estimated human daily intakes
FAO	Food and Agricultural Organization
GC×GC-µECD	Comprehensive two-dimensional gas chromatography with a micro electron-capture detector
GC × GC–TOF-MS	A comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry
GC-LRMS/MS	Gas Chromatography/Low Resolution Mass Spectrometry/ Mass Spectrometry
GC-HRMS	Gas Chromatography/ High Resolution Mass Spectrometr
IARC	International Agency for Research on Cancer
JECFA	Joint FAO/WHO Expert Committee on Food Additives and Contaminants
IPR	Initial Precision and Recovery
KDEM	Kinetic Dietary Exposure Model
KFDA	Korea Food and Drug Administration
LMCO	laccase-like multi-copper oxidase
LLE	Liquid-Liquid Extraction
LOD	Limit of detection
LOQ	Limit of quantitation
LOAEL	The lowest observed adverse effect level
MWI	Municipal Waste Incinerator
ND	Not detected

LIST OF ABBREVIATIONS

Abbreviate	Description in details
OLS	The ordinary least squares regressions
OPR	Ongoing Precision and Recovery
PAHs	Polycyclic Aromatic Hydrocarbons
PBDE	Poly Brominated Diphenyl Ether
PCA	Principal Component Analysis
PCDDs	Polychlorinated dibenzo-p-dioxins
PCDFs	Polychlorinated dibenzofurans
PLE	Pressurized liquid extraction
PMTMI	Provisional maximum tolerable monthly intake
ppt	Part per trillion
POPs	Persistent Organic Pollutants
PTMI	Provisional tolerable maximum monthly intake
PTs	proficiency tests
PVC	Polyvinyl chloride
QA/QC	Quality assurance/quality control
REP	Relative potency value
SRw,rel	Relative intermediate precision standard deviation
RSD	Relative standard deviation
SCF	Scientific Committee on Food
SCWE	Subcritical water extraction
SPE	Solid phase extraction
TCDD	Tetrachloro dibenzo-p-dioxin
TDI	Total Daily Intake
TDS	Total Diet Study
TEFs	Toxic equivalency factors
TEQs	Toxic equivalency values
TMI	Tolerable monthly intake
TWI	Tolerable weekly intake
USEPA	United State of Environmental Protection Agency
WHO	World Health Organization