Journal of Toxicology

Environmental Pollution and Food Safety: Health Hazard Analysis and Human Health Risk Assessment

Lead Guest Editor: Wageh Sobhy Darwish
Guest Editors: Hazuki Mizukawa, Rialet Pieters, Emmanuel T. Ogbomida, and Lesa Thompson

Environmental Pollution and Food Safety: Health Hazard Analysis and Human Health Risk Assessment

Journal of Toxicology

Environmental Pollution and Food Safety: Health Hazard Analysis and Human Health Risk Assessment

Lead Guest Editor: Wageh Sobhy Darwish
Guest Editors: Hazuki Mizukawa, Rialet Pieters, Emmanuel T. Ogbomida, and Lesa Thompson

Contents

Hindawi
Journal of Toxicology
Volume 2019, Article ID 9246495, 24 pages
https://doi.org/10.1155/2019/9246495

Research Article

An Adverse Outcome Pathway Linking Organohalogen Exposure to Mitochondrial Disease

Brooke McMinn, Alicia L. Duval, and Christie M. Sayes

Department of Environmental Science, Baylor University, One Bear Place # 97266, Waco, TX 76798-7266, USA

Correspondence should be addressed to Christie M. Sayes; christie_sayes@baylor.edu

Received 24 August 2018; Accepted 5 March 2019; Published 1 April 2019

Guest Editor: Lesa Thompson

Adverse outcome pathways (AOPs) are pragmatic tools in human health hazard characterization and risk assessment. As such, one of the main goals of AOP development is to provide a clear, progressive, and linear mechanistic representation of pertinent toxicological key events (KEs) occurring along the different levels of biological organization. Here, we present an AOP framework that depicts how exposure to organohalogens can lead to mitochondrial disease. Organohalogens are disinfectant by-products (DBPs) found in our drinking water. Chloroform, trichloroacetic acid, and trichlorophenol were selected to represent specific types of organohalogens for the development of this AOP. Although each of these compounds contains chlorine atoms, they differ in aromaticity and solubility, which have a significant impact on their potency. This AOP consists of two main pathways, both of which are triggered by the molecular initiating event (MIE) of excessive reactive oxygen species generation. Pathway 1 details the downstream consequences of oxidative stress, which include mitochondrial DNA damage, protein aggregation, and depolarization of the mitochondrial membrane. Pathway 2 shows the KEs that result from inadequate supply of glutathione, including calcium dysregulation and ATP depletion. Pathways 1 and 2 converge at a common KE: opening of the mitochondrial membrane transition pore (mPTP). This leads to the release of cytochrome c, caspase activation, apoptosis, and mitochondrial disease. This AOP was developed according to the Organisation for Economic Co-operation and Development guidance, including critical consideration of the Bradford Hill criteria for Weight of Evidence assessment and key questions for evaluating confidence. The presented AOP is expected to serve as the basis for designing new toxicological tests as well as the characterization of novel biomarkers for disinfectant by-product exposure and adverse health effects.

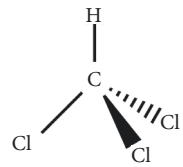
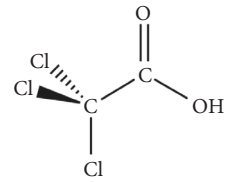
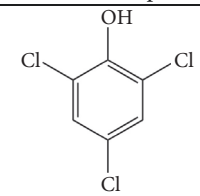
1. Introduction

In the early 1960s, researchers identified mitochondrial disease as a serious clinical condition and have since increased their efforts to identify its etiology [1]. Mitochondrial diseases are progressive, chronic, and irreversible illnesses that result from failure of mitochondrial organelles, which are specialized cellular compartments responsible for generating more than 90% of the energy required to sustain life. In general, mitochondrial failure leads to cell injury, closely followed by cellular demise [2]. When multiple cells expire through this pathway, the most common adverse outcome (AO) is organ failure [3–6].

With the exception of red blood cells, all human cells contain mitochondria. Therefore, mitochondrial dysfunction can occur in nearly any organ system of the human body and cause a variety of adverse health conditions, ranging from mild (i.e., nausea or mild cognitive impairment) to severe (heart failure or Parkinson's Disease) [7–9]. The most common symptoms of mitochondrial dysfunction include loss of muscle coordination, muscle weakness, developmental delays, learning disabilities, heart disease, diabetes, gastrointestinal disorders, liver disease, kidney disease, and neurological problems [10–14].

Mitochondrial dysfunction becomes mitochondrial disease as soon as mutations caused by xenobiotic exposure, genetics, or a combination thereof are identified. Mutation markers can be found in either mitochondrial or nuclear DNA [15–17]. While most cases of mitochondrial disease are linked to a genetic malfunction, substantial evidence published in the recent literature suggests that environmental triggers or exposure to certain xenobiotics may be responsible

Table 1: Organohalogen characterization table. The table includes physicochemical properties of three primary disinfectant by-products: chloroform, chloroacetic acid, and chlorophenol. The LD_{50} values of the materials after oral exposure to rats are also included. These organohalogens are known as mitochondrial toxins and potent disruptors of mitochondrial respiratory chain.

	Chloroform	Chloroacetic acid	Chlorophenol
IUPAC name	Trichloromethane	Trichloroacetic acid	2,4,6-Trichlorophenol
Chemical structure			
CAS number	67-66-3	76-03-9	88-06-2
Chemical formula	$CHCl_3$	$C_2HCl_3O_2$	$C_6H_3Cl_3O$
Molecular weight	119.37 g/mol	163.38 g/mol	197.45 g/mol
Density	1.489 g/cm^3 (20°C)	1.63 g/cm^3 (20°C)	1.675 g/cm^3 (20°C)
Water solubility	8.09 g/L (20°C)	>10,000 g/L (20°C)	20.0 g/L (20°C)
Dissociation (pKa)	15.7 (20°C)	0.66 (20°C)	8.56 (20°C)
Boiling point	61.2°C	195.5°C	246°C
Toxicity LD_{50} (rats,oal)	695 mg/kg [51–54]	425 mg/kg [55]	670 mg/kg [56–59]

for the onset of some mitochondrial diseases [18, 19]. Exposure to pesticides is one environmental trigger that is frequently cited in the literature [20–22]. Ingestion of disinfectant by-products (DBPs) from drinking water may also be a trigger of mitochondrial disease. Risk assessments performed on these environmental exposures provide evidence for addressing this growing public health concern [23–27].

Disinfectant products, such as chlorine or chloramine, are added to the drinking water supply to mitigate the onset of waterborne illnesses due to microorganisms. While this technology has greatly improved water quality around the world, the addition of chlorinated compounds to water has produced an unintended consequence: unnatural disinfectant by-products (DBPs) littering plumbing within the water distribution system (Table 1). Chlorine reacts readily with water constituents (such as metal ions and carbonaceous species) to produce DBPs, which have been associated with a variety of human health effects, including cancer [28–33]. A substantial amount of research has been performed investigating the chemical mechanisms of formation and subsequent compound identification [34–37]. However, fewer studies have reported the toxicological mechanisms of action after DBP exposure to humans [38–40]. The chemical mechanisms of DBP formation aid in exposure analyses, but, without adequate toxicological mechanisms of action reported, risk assessments are difficult to perform.

Toxicologists face many challenges when performing human risk assessments, such as incomplete dosimetry information, disparate *in vitro* and *in vivo* hazard results, and lack of human epidemiological data [41–43]. There is an ever-increasing number of substances (i.e. chemicals, particles, aerosols, pharmaceuticals, advanced materials, and by-products) that should be tested for toxicity, evaluated for exposure, and assessed for risk. Unfortunately, insufficient resources make these thorough analyses difficult to perform in a timely and cost-effective manner [44, 45]. Additionally, there is pressure to reduce the use of animals used in toxicological analyses [46]. Therefore, it is increasingly necessary to use available data from the literature to design, collect, and interpret new toxicological studies that answer unresolved questions or gaps in data [47, 48].

One promising pathway-based analysis tool is adverse outcome pathway (AOP) development (Figure 1). An AOP describes the progression of adverse health effects from lower-level molecular reactions to higher-level disease onset [47, 49]. AOP development begins with identifying a molecular initiating event (MIE) and ultimately concludes by recognizing the adverse outcome(s) (AO) of regulatory significance. The MIE and AO are related via a sequence of biologically plausible and scientifically supported key events (KEs) of increasing complexity. The relationships between the KEs are activated through structural and functional relationships coupled with weight of evidence criteria [50].

The purpose of this manuscript is to explain and support a developed AOP that relates a global environmental exposure (ingestion of organohalogens) to mitochondrial dysfunction (opening of the mitochondrial permeability transition pore, mPTP) using individual mechanistic events identified in the peer-reviewed literature (Figure 2). This AOP has the potential to serve the scientific community as a basis for the development of new and targeted toxicological tests as well as the characterization of novel biomarkers of oxidative stress-induced organ system dysfunction.

2. Methods

2.1. AOP Development. There is no single strategy established that is suitable for all AOP development scenarios. Instead,

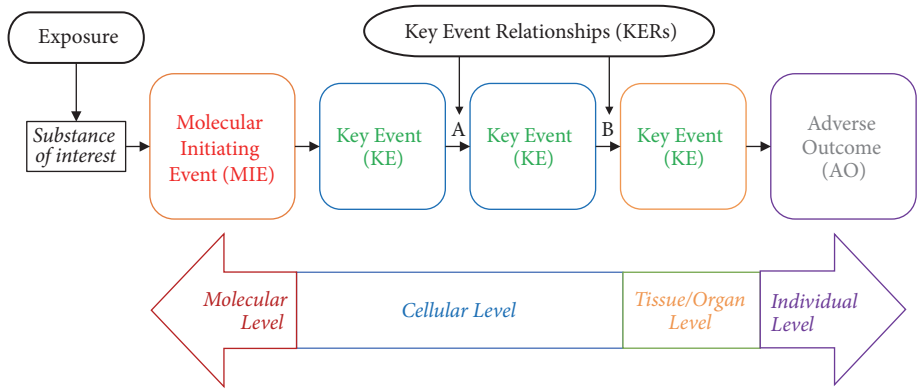


Figure 1: A schematic representation of the adverse outcome pathway (AOP). An AOP starts with a molecular initiating event (MIE) which is triggered by exposure to a substance of interest that interacts or reacts with a biological target, often a molecule. This MIE leads to a sequential series of intermediate key events (KE) along the different levels of biological organization to produce an adverse outcome (AO). A and B represent the relationships between two unique key events (KERs).

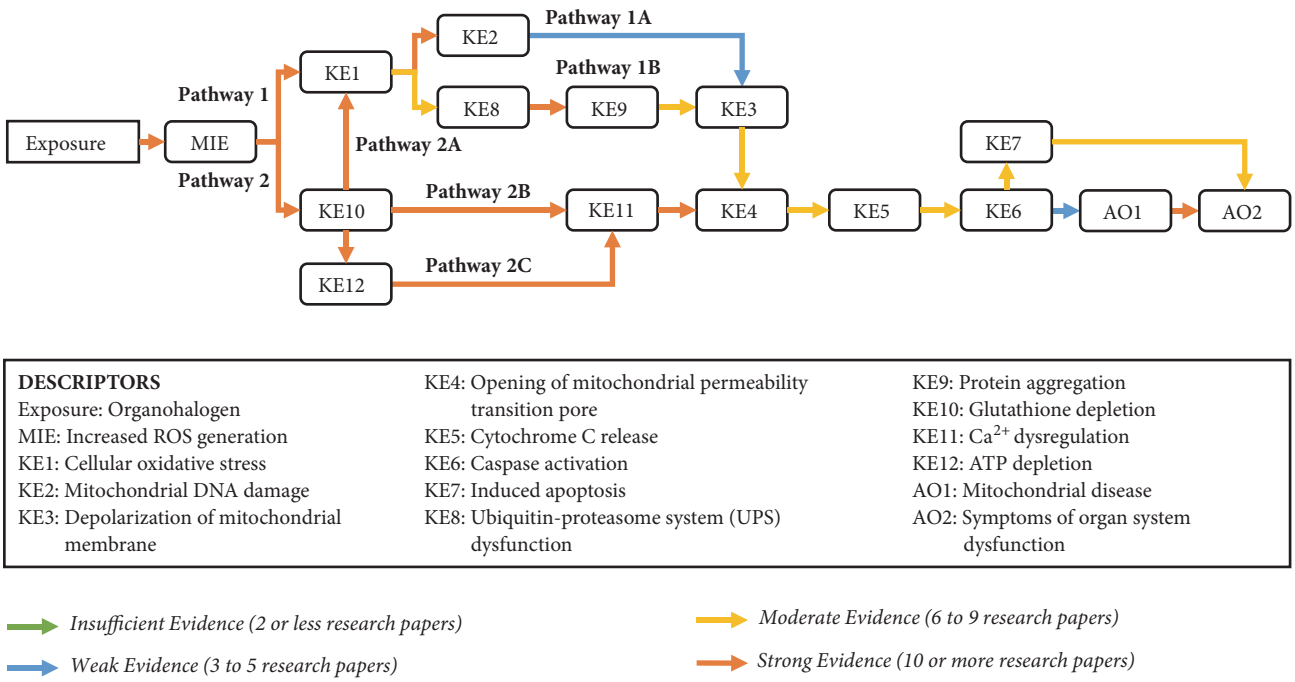


Figure 2: Weight of evidence assessment of key events (KE) and key event relationships supporting this AOP. All substances of interest (chloroform, chloroacetic acid, and chlorophenol) activate the proposed pathways.

AOP developers use different strategies to develop a single or network of pathways leading to adverse outcomes. The strategy most often utilized during the early stages of AOP development is data-mining [49]. This process includes analysis of relevant literature and database mining approaches to infer relationships between KEs (a.k.a, KERs). Mitochondrial disease AOPs are in their infancy; therefore, we used a combination of keyword searches. We conducted an extensive literature search using a combination of the following terms: "(mitochondrial∗) AND (AOP OR adverse outcome pathway)". The primary databases searched were PubMed, Web of Science, and Scopus. The search results were not restricted by a date range; however, most papers included in this analysis were published within the last 20 years (1998-2018). A total of 70 unique papers were returned with these keyword phrases. On the other hand, papers linking "(mitochondrial∗) AND (environment∗)" are plentiful. A total of 15,221 unique papers were returned with these keywords. The next section describes the screening of these papers in an effort to narrow the developed AOP.

2.2. AOP Representation and Evaluation. The search terms "mitochondrial" AND "environmental" produced too much data to mine AOP key events and their associated relationships. From this initial search, we screened abstracts using specific terms related to different phases with in the AOP:

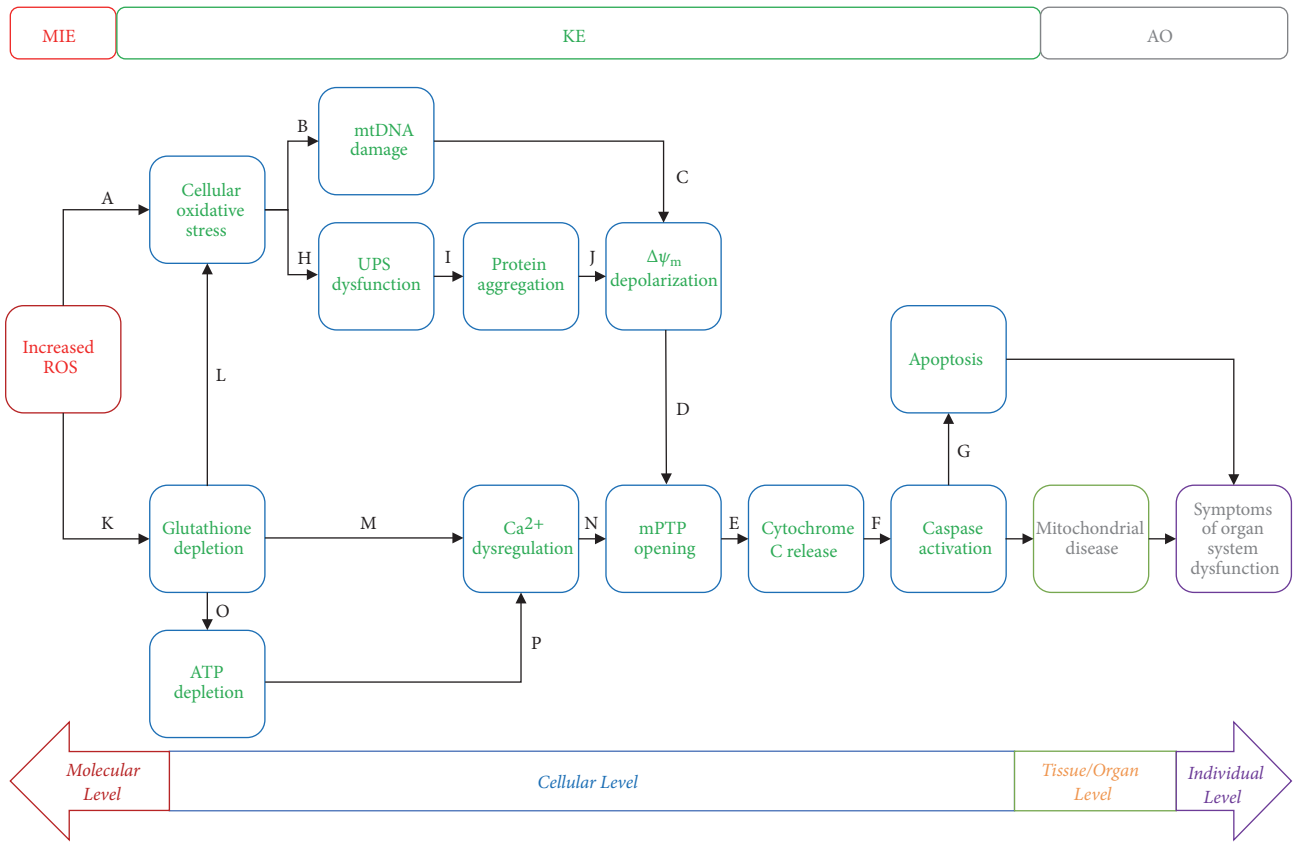


Figure 3: Graphical representation of organohalogen exposure to mitochondrial disease AOP. Key events at the molecular and cellular levels lead to AOs at the tissue/organ and individual levels. Exposure to organohalogens cause increased ROS production (Molecular Initiating Event, MIE), triggering the key events (KEs) that result in the adverse outcome (AO), defined as the onset of mitochondrial disease and the associated symptoms of organ symptom dysfunction. This AOP depicts 2 distinct pathways from MIE to AO.

(1) First, we included "organohalogen" and eliminated other environmental factors to build the molecular level.

(2) Second, we included "mitochondria", "oxidative stress", and "glutathione" to build the cellular level.

(3) Third, we included "mitochondrial permeability", "caspase activation", "apoptosis" to develop the organ level. Articles that focused on mitochondrial dysfunction and genetic causes were excluded.

Upon applying inclusionary and exclusionary criteria, we were left with a total of 189 unique eligible articles. This process ensured that the articles with the most relevance were included in the final AOP.

Appropriate KEs and their relationships were reported using a flow diagram showing the key event relationships (KERs) along the increasingly complicated levels of biological organization (i.e., molecular, cellular, tissue/organ, and individual levels) in a consecutive manner (Figure 1). Connections between events were decided based on a strength-based weight-of-evidence (WoE) assessment of the MIE, KE, and AO linkages (Figure 3). A final evaluation was conducted in two steps. First, Bradford Hill criteria were used to assess the WoE of the AOP to establish a causal link between the different blocks representing biological organization (Table 2). Second, we reported the confidence associated with each causal link by addressing OECD's proposed questions (Figures 4, 5, 6, and 7). This evaluation process ensures that the resultant AOP meets the minimal information requirements to establish plausibility of the proposed pathway.

3. Results

3.1. Weight of Evidence

3.1.1. Empirical Support of the KERs. Based on well-established knowledge of mitochondrial function, the biological plausibility between increased ROS production and mitochondrial disease is strong. This is often associated with mitochondrial electron transport chain disruption or complex I inhibition [60–62]. Figure 2 contains the details for the biological plausibility of the KERs.

The degree of empirical support for the KERs ranges from *insufficient* to *strong* evidence. Organohalogens produce ROS species in water [63–67]. Many of these tri-chlorine containing compounds have a high affinity for disruption of mitochondrial electron transport chain (ETC) [63, 65]. Table 1

Table 2: Organohalogen AOP References. The predominate pathways create a network of molecular events triggered during the development of mitochondrial disease. The key event relationships between each individual key event are presented here in tabular form.

Molecular Initiating Event	
Chloroform ⟶ increased ROS	(1) Chiu, C.-H., et al. Chloroform extract of solanum lyratum induced G0/G1 arrest via p21/p16 and induced apoptosis via reactive oxygen species, caspases and mitochondrial pathways in human oral cancer cell lines. *The American journal of Chinese medicine* 43.07 (2015): 1453. (2) Zhang, Y., et al. Chemical compositions and antiproliferation activities of the chloroform fraction from *P. fomentarius* in K562 cells. *Human & experimental toxicology* 34.7 (2015): 732. (3) Wang, Y., et al. Investigating migration inhibition and apoptotic effects of Fomitopsis pinicola chloroform extract on human colorectal cancer SW-480 cells. *PloS one* 9.7 (2014): e101303. (4) Looi, C.Y., et al. Induction of apoptosis in melanoma A375 cells by a chloroform fraction of *Centratherum anthelminticum (L.)* seeds involves NF-kappaB, p53 and Bcl-2-controlled mitochondrial signaling pathways. *BMC complementary and alternative medicine* 13.1 (2013): 166. (5) Faustino-Rocha, A.I., et al. Trihalomethanes in liver pathology: Mitochondrial dysfunction and oxidative stress in the mouse. *Environmental toxicology* 31.8 (2016): 1009. (6) Ali, A., et al. Effect of drinking water disinfection by-products in human peripheral blood lymphocytes and sperm. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 770 (2014): 136. (7) Brunner, E.A., et al. Effects of anesthesia on intermediary metabolism. *Annual review of medicine* 26.1 (1975): 391.
Chloroform ⟶ decreased ATP	(1) Faustino-Rocha, A. I., et al. Trihalomethanes in liver pathology: Mitochondrial dysfunction and oxidative stress in the mouse. *Environmental toxicology* 31.8 (2016): 1009. (2) Rottenberg, H. Uncoupling of oxidative phosphorylation in rat liver mitochondria by general anesthetics. *Proceedings of the National Academy of Sciences* 80.11 (1983): 3313. (3) Brunner, E.A., et al. Effects of anesthesia on intermediary metabolism. *Annual review of medicine* 26.1 (1975): 391.
Chloroform ⟶ decreased glutathione (GSH)	(1) Ekström, T., et al. Chloroform-induced glutathione depletion and toxicity in freshly isolated hepatocytes. *Biochemical pharmacology* 29.22 (1980): 3059. (2) Docks, E. L., et al. The role of glutathione in chloroform-induced hepatotoxicity. *Experimental and molecular pathology* 24.1 (1976): 13. (3) Beddowes, E.J., et al. Chloroform, carbon tetrachloride and glutathione depletion induce secondary genotoxicity in liver cells via oxidative stress. *Toxicology* 187.2-3 (2003): 101. (4) Wang, Y., et al. Investigating migration inhibition and apoptotic effects of Fomitopsis pinicola chloroform extract on human colorectal cancer SW-480 cells. *PloS one* 9.7 (2014): e101303. (5) Abbassi, R., et al. Chloroform-induced oxidative stress in rat liver: implication of metallothionein. *Toxicology and industrial health* 26.8 (2010): 487. (6) Hewitt, W.R., et al. Nephrotoxic interactions between ketonic solvents and halogenated aliphatic chemicals. *Toxicological Sciences* 4.6 (1984): 902. (7) Skrzypińska-Gawrysiak, M., et al. The hepatotoxic action of chloroform: short-time dynamics of biochemical alterations and dose-effect relationships. *Polish journal of occupational medicine and environmental health* 4.1 (1991): 77. (8) Azri-Meehan, S., et al. The hepatotoxicity of chloroform in precision-cut rat liver slices. *Toxicology* 73.3 (1992): 239. (9) Ekström, T., et al. Lipid peroxidation in vivo monitored as ethane exhalation and malondialdehyde excretion in urine after oral administration of chloroform. *Basic & Clinical Pharmacology & Toxicology* 58.4 (1986): 289. (10) Qin, L.-Q., et al. One-day dietary restriction changes hepatic metabolism and potentiates the hepatotoxicity of carbon tetrachloride and chloroform in rats. *The Tohoku journal of experimental medicine* 212.4 (2007): 379. (11) Cohen, P.J., et al. Continuous in vivo measurement of hepatic lipoperoxidation using chemiluminescence: halothane and chloroform compared. *Anesthesia and analgesia* 70.3 (1990): 296.

Table 2: Continued.

Chlorophenol ⟶ increased ROS	(1) Luo, Y., et al. 2-Chlorophenol induced hydroxyl radical production in mitochondria in Carassius auratus and oxidative stress–An electron paramagnetic resonance study. *Chemosphere* 71.7 (2008): 1260. (2) Luo, Y., et al. 2-Chlorophenol induced ROS generation in fish Carassius auratus based on the EPR method. *Chemosphere* 65.6 (2006): 1064. (3) Khachatryan, L., et al. Environmentally persistent free radicals (EPFRs). 1. Generation of reactive oxygen species in aqueous solutions. *Environmental science & technology* 45.19 (2011): 8559. (4) Atkinson, A., et al. Increased oxidative stress in the liver of mice treated with trichloroethylene. *Biochemistry and molecular biology international* 31.2 (1993): 297. (5) Igbinosa, E.O., et al. Toxicological profile of chlorophenols and their derivatives in the environment: the public health perspective. *The Scientific World Journal* (2013). (6) Bukowska, B., et al. Comparison of the effect of phenol and its derivatives on protein and free radical formation in human erythrocytes (in vitro). *Blood Cells, Molecules, and Diseases* 39.3 (2007): 238. (7) Michalowicz, J., et al. The Effects of 2, 4, 5-Trichlorophenol on Some Antioxidative Parameters and the Activity of Glutathione S-Transferase in Reed Canary Grass Leaves (Phalaris arudinacea). *Polish Journal of Environmental Studies* 18.5 (2009). (8) Li, Z., et al. Stress responses to trichlorophenol in Arabidopsis and integrative analysis of alteration in transcriptional profiling from microarray. *Gene* 555.2 (2015): 159. (9) Li, F., et al. Hydroxyl radical generation and oxidative stress in Carassius auratus liver as affected by 2, 4, 6-trichlorophenol. *Chemosphere* 67.1 (2007): 13. (10) Xia, Xi., et al. Response of selenium-dependent glutathione peroxidase in the freshwater bivalve Anodonta woodiana exposed to 2, 4-dichlorophenol, 2, 4, 6-trichlorophenol and pentachlorophenol. *Fish & shellfish immunology* 55 (2016): 499. (11) Dong, Y.-L., et al. Induction of oxidative stress and apoptosis by pentachlorophenol in primary cultures of Carassius carassius hepatocytes. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 150.2 (2009): 179.
Chlorophenol ⟶ decreased ATP	(1) Aschmann, C., et al. Short-term effects of chlorophenols on the function and viability of primary cultured rat hepatocytes. *Archives of toxicology* 63.2 (1989): 121. (2) Stockdale, M., et al. Effects of ring substituents on the activity of phenols as inhibitors and uncouplers of mitochondrial respiration. *The FEBS Journal* 21.4 (1971): 565. (3) Mitsuda, H., et al. Effect of chlorophenol analogues on the oxidative phosphorylation in rat liver mitochondria. *Agricultural and Biological Chemistry* 27.5 (1963): 366. (4) Stockdale, M., et al. Influence of ring substituents on the action of phenols on some dehydrogenases, phosphokinases and the soluble ATPase from mitochondria. *The FEBS Journal* 21.3 (1971): 416. (5) Hugül, M., et al. Modeling the kinetics of UV/hydrogen peroxide oxidation of some mono-, di-, and trichlorophenols. *Journal of hazardous materials* 77.1-3 (2000): 193. (6) Juhl, U., et al. The Induction of Dna Srtrand Breaks and Formation of Semiquinone Radicals by Metabolites of 2, 4, 5-Trichlorophenol. *Free radical research communications* 11.6 (1991): 295.
Chlorophenol ⟶ decreased glutathione	(1) Li, F., et al. Hydroxyl radical generation and oxidative stress in Carassius auratus liver as affected by 2, 4, 6-trichlorophenol. *Chemosphere* 67.1 (2007): 13. (2) Dong, Y.-L., et al. Induction of oxidative stress and apoptosis by pentachlorophenol in primary cultures of Carassius carassius hepatocytes. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* 150.2 (2009): 179. (3) Wang, Y.-J., et al. Induction of glutathione depletion, p53 protein accumulation and cellular transformation by tetrachlorohydroquinone, a toxic metabolite of pentachlorophenol. *Chemico-biological interactions* 105.1 (1997): 1. (4) Valentovic, M., et al. 2-Amino-5-chlorophenol toxicity in renal cortical slices from Fischer 344 rats: effect of antioxidants and sulfhydryl agents. *Toxicology and applied pharmacology* 161.1 (1999): 1. (5) Luo, Y., et al. 2-Chlorophenol induced hydroxyl radical production in mitochondria in Carassius auratus and oxidative stress–An electron paramagnetic resonance study. *Chemosphere* 71.7 (2008): 1260. (6) Götz, R., et al. Effects of pentachlorophenol and 2, 4, 6-trichlorophenol on the disposition of sulfobromophthalein and respiration of isolated liver cells. *Archives of toxicology* 44.1-3 (1980): 147. (7) Ahammed, G.J., et al. 24-Epibrassinolide alleviates organic pollutants-retarded root elongation by promoting redox homeostasis and secondary metabolism in Cucumis sativus L. *Environmental Pollution* 229 (2017): 922.

Table 2: Continued.

Chloroacetic acid ⟶ increased ROS	(1) Lu, T.-H., et al. Chloroacetic acid triggers apoptosis in neuronal cells via a reactive oxygen species-induced endoplasmic reticulum stress signaling pathway. *Chemico-biological interactions* 225 (2015): 1. (2) Chen, C.-H., et al. Chloroacetic acid induced neuronal cells death through oxidative stress-mediated p38-MAPK activation pathway regulated mitochondria-dependent apoptotic signals. *Toxicology* 303 (2013): 72. (3) Pals, J., et al. Human cell toxicogenomic analysis linking reactive oxygen species to the toxicity of monohaloacetic acid drinking water disinfection byproducts. *Environmental science & technology* 47.21 (2013): 12514. (4) Pals, J., et al. Biological mechanism for the toxicity of haloacetic acid drinking water disinfection byproducts. *Environmental science & technology* 45.13 (2011): 5791. (5) Yin, J., et al. Comparative toxicity of chloro-and bromo-nitromethanes in mice based on a metabolomic method. *Chemosphere* 185 (2017): 20. (6) Ali, A., et al. Effect of drinking water disinfection by-products in human peripheral blood lymphocytes and sperm. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 770 (2014): 136. (7) Marsà, A., et al. Hazard assessment of three haloacetic acids, as byproducts of water disinfection, in human urothelial cells. *Toxicology and applied pharmacology* 347 (2018): 70. (8) Zhang, X., et al. 2, 4, 6-Trichlorophenol cytotoxicity involves oxidative stress, endoplasmic reticulum stress, and apoptosis. *International journal of toxicology* 33.6 (2014): 532. (9) Celik, I., et al. Hepatoprotective role and antioxidant capacity of pomegranate (Punica granatum) flowers infusion against trichloroacetic acid-exposed in rats. *Food and Chemical Toxicology* 47.1 (2009): 145. (10) Dad, A., et al. Pyruvate remediation of cell stress and genotoxicity induced by haloacetic acid drinking water disinfection by-products. *Environmental and molecular mutagenesis* 54.8 (2013): 629. (11) Dad, A., et al. Haloacetic Acid Water Disinfection Byproducts Affect Pyruvate Dehydrogenase Activity and Disrupt Cellular Metabolism. *Environmental science & technology* 52.3 (2018): 1525.
Chloroacetic acid ⟶ decreased ATP	(1) Dad, A., et al. Pyruvate remediation of cell stress and genotoxicity induced by haloacetic acid drinking water disinfection by-products. *Environmental and molecular mutagenesis* 54.8 (2013): 629. (2) Dad, A., et al. Haloacetic Acid Water Disinfection Byproducts Affect Pyruvate Dehydrogenase Activity and Disrupt Cellular Metabolism. *Environmental science & technology* 52.3 (2018): 1525. (3) Schmidt, M., et al. Effects of chlorinated acetates on the glutathione metabolism and on glycolysis of cultured astrocytes. *Neurotoxicity research* 19.4 (2011): 628.
Chloroacetic acid ⟶ decreased glutathione	(1) Chen, C.-H., et al. Chloroacetic acid induced neuronal cells death through oxidative stress-mediated p38-MAPK activation pathway regulated mitochondria-dependent apoptotic signals. *Toxicology* 303 (2013): 72. (2) Schmidt, M., et al. Effects of chlorinated acetates on the glutathione metabolism and on glycolysis of cultured astrocytes. *Neurotoxicity research* 19.4 (2011): 628. (3) Lu, T.-H., et al. Chloroacetic acid triggers apoptosis in neuronal cells via a reactive oxygen species-induced endoplasmic reticulum stress signaling pathway. *Chemico-biological interactions* 225 (2015): 1. (4) Bruschi, S., et al. In vitro cytotoxicity of mono-, di-, and trichloroacetate and its modulation by hepatic peroxisome proliferation. *Fundamental and Applied Toxicology* 21.3 (1993): 366.
Pathway 1A & Pathway 1B	
MIE ⟶ KE1 Increased ROS generation ⟶ oxidative stress	(1) Sun, F., et al. Environmental neurotoxic chemicals-induced ubiquitin proteasome system dysfunction in the pathogenesis and progression of Parkinson's disease. *Pharmacology & therapeutics* 114.3 (2007): 327. (2) Bender, A., et al. TOM40 mediates mitochondrial dysfunction induced by α-synuclein accumulation in Parkinson's disease. *PloS one* 8.4 (2013): e62277. (3) Hauser, D.N., et al. Mitochondrial dysfunction and oxidative stress in Parkinson's disease and monogenic parkinsonism. *Neurobiology of disease* 51 (2013): 35. (4) Mikhed, Y., et al. Mitochondrial oxidative stress, mitochondrial DNA damage and their role in age-related vascular dysfunction. *International journal of molecular sciences* 16.7 (2015): 15918. (5) Zhang, W., et al. Mediating effect of ROS on mtDNA damage and low ATP content induced by arsenic trioxide in mouse oocytes. *Toxicology in Vitro* 25.4 (2011): 979.

Table 2: Continued.

Pathway 1A	
KE1 ⟶ KE2 Oxidative stress causes mtDNA damage	(1) Bender, A., et al. TOM40 mediates mitochondrial dysfunction induced by α-synuclein accumulation in Parkinson's disease. *PloS one* 8.4 (2013): e62277. (2) Mikhed, Y., et al. Mitochondrial oxidative stress, mitochondrial DNA damage and their role in age-related vascular dysfunction. *International journal of molecular sciences* 16.7 (2015): 15918. (3) Zhang, W., et al. Mediating effect of ROS on mtDNA damage and low ATP content induced by arsenic trioxide in mouse oocytes. *Toxicology in Vitro* 25.4 (2011): 979. (4) Ayala-Peña, S. Role of oxidative DNA damage in mitochondrial dysfunction and Huntington's disease pathogenesis. *Free Radical Biology and Medicine* 62 (2013): 102. (5) Birch-Machin, M.A., et al. Mitochondrial DNA damage as a biomarker for ultraviolet radiation exposure and oxidative stress. *British Journal of Dermatology* 169.s2 (2013): 9. (6) Santos, R.X., et al. Mitochondrial DNA oxidative damage and repair in aging and Alzheimer's disease. *Antioxidants & redox signaling* 18.18 (2013): 2444. (7) Yue, R., et al. Mitochondrial DNA oxidative damage contributes to cardiomyocyte ischemia/reperfusion-injury in rats: cardioprotective role of lycopene. *Journal of cellular physiology* 230.9 (2015): 2128. (8) Han, Y., et al. Oxidative stress induces mitochondrial DNA damage and cytotoxicity through independent mechanisms in human cancer cells. *BioMed research international* 2013 (2013). (9) Chan, S.W., et al. Simultaneous quantification of mitochondrial DNA damage and copy number in circulating blood: a sensitive approach to systemic oxidative stress. *BioMed research international* 2013 (2013). (10) Wei, Y.-H. Mitochondrial DNA mutations and oxidative damage in aging and diseases: an emerging paradigm of gerontology and medicine. *Proceedings of the National Science Council, Republic of China. Part B, Life sciences* 22.2 (1998): 55. (11) Kim, Y.J., et al. Cytoplasmic ribosomal protein S3 (rpS3) plays a pivotal role in mitochondrial DNA damage surveillance. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research* 1833.12 (2013): 2943. (12) Basu, S., et al. Transcriptional mutagenesis by 8-oxodG in α-synuclein aggregation and the pathogenesis of Parkinson's disease. *Experimental & molecular medicine* 47.8 (2015): e179.
KE2⟶ KE3 mtDNA damage causes depolarization of mitochondrial membrane	(1) Kim, S.J., et al. The role of mitochondrial DNA in mediating alveolar epithelial cell apoptosis and pulmonary fibrosis. *International journal of molecular sciences* 16.9 (2015): 21486. (2) Santos, J.H., et al. Cell sorting experiments link persistent mitochondrial DNA damage with loss of mitochondrial membrane potential and apoptotic cell death. *Journal of biological chemistry* 278.3 (2003): 1728. (3) Ehlers, R.A., et al. Mitochondrial DNA damage and altered membrane potential ($\Delta\Psi$) in pancreatic acinar cells induced by reactive oxygen species. *Surgery* 126.2 (1999): 148.
KE3⟶KE4 Depolarization of mitochondrial membrane ⟶ opening of mPTP	(1) Bernardi, P. Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore by the proton electrochemical gradient. Evidence that the pore can be opened by membrane depolarization. *Journal of Biological Chemistry* 267.13 (1992): 8834. (2) Bernardi, P. Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore by the proton electrochemical gradient. Evidence that the pore can be opened by membrane depolarization. *Journal of Biological Chemistry* 267.13 (1992): 8834. (3) Petronilli, V., et al. Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore. II. The minimal requirements for pore induction underscore a key role for transmembrane electrical potential, matrix pH, and matrix Ca2+. *Journal of Biological Chemistry* 268.2 (1993): 1011. (4) Ly, J.D., et al. The mitochondrial membrane potential ($\Delta\psi$m) in apoptosis; an update. *Apoptosis* 8.2 (2003): 115. (5) Scorrano, L., et al. On the voltage dependence of the mitochondrial permeability transition pore A critical appraisal. *Journal of Biological Chemistry* 272.19 (1997): 12295. (6) Petronilli, V., et al. The voltage sensor of the mitochondrial permeability transition pore is tuned by the oxidation-reduction state of vicinal thiols. Increase of the gating potential by oxidants and its reversal by reducing agents. *Journal of Biological Chemistry* 269.24 (1994): 16638.

Table 2: Continued.

KE4⟶KE5 Opening of mPTP ⟶ cytochrome c release	(1) Yang, B.-C., et al. Crotonaldehyde induces apoptosis in alveolar macrophages through intracellular calcium, mitochondria and p53 signaling pathways. *The Journal of toxicological sciences* 38.2 (2013): 225. (2) Yamamoto, T., et al. The mechanisms of the release of cytochrome C from mitochondria revealed by proteomics analysis. *Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan* 132.10 (2012): 1099. (3) Tornero, D., et al. The role of the mitochondrial permeability transition pore in neurodegenerative processes. *Revista de neurologia* 35.4 (2002): 354. (4) Song, T., et al. Protection effect of atorvastatin in cerebral ischemia-reperfusion injury rats by blocking the mitochondrial permeability transition pore. *Genet Mol Res* 13.4 (2014): 10632. (5) Ma, X.D., et al. Mechanism of opening of mitochondrial permeability transition pore induced by arsenic trioxide. *Ai zheng Aizheng Chinese journal of cancer* 25.1 (2006): 17. (6) Crompton, Martin. The mitochondrial permeability transition pore and its role in cell death. *Biochemical Journal* 341.2 (1999): 233. (7) Ou, Z., et al. Mitochondrial-dependent mechanisms are involved in angiotensin II-induced apoptosis in dopaminergic neurons. *Journal of the renin-angiotensin-aldosterone system* 17.4 (2016): 1470320316672349. (8) Raza, H., et al. Acetylsalicylic acid-induced oxidative stress, cell cycle arrest, apoptosis and mitochondrial dysfunction in human hepatoma HepG2 cells. *European journal of pharmacology* 668.1-2 (2011): 15.
KE5⟶KE6 Cytochrome c release ⟶ activation of caspases	(1) Maria, D.A., et al. A novel proteasome inhibitor acting in mitochondrial dysfunction, ER stress and ROS production. *Investigational new drugs* 31.3 (2013): 493. (2) Spano, M., et al. The possible involvement of mitochondrial dysfunctions in Lewy body dementia: a systematic review. *Functional neurology* 30.3 (2015): 151. (3) Hashimoto, M., et al. Role of protein aggregation in mitochondrial dysfunction and neurodegeneration in Alzheimer's and Parkinson's diseases. *Neuromolecular medicine* 4.1-2 (2003): 21. (4) Kakimura, J.-I., et al. Release and aggregation of cytochrome c and α-synuclein are inhibited by the antiparkinsonian drugs, talipexole and pramipexole. *European journal of pharmacology* 417.1-2 (2001): 59. (5) Jiang, X., et al. Cytochrome C-mediated apoptosis. *Annual review of biochemistry* 73 (2004). (6) Garcia, M., et al. Mitochondria, motor neurons and aging. *Journal of the neurological sciences* 330.1 (2013): 18. (7) Lu, C., et al. Neuroprotective effects of tetramethylpyrazine against dopaminergic neuron injury in a rat model of Parkinson's disease induced by MPTP. *International journal of biological sciences* 10.4 (2014): 350. (8) Raza, H., et al. Acetylsalicylic acid-induced oxidative stress, cell cycle arrest, apoptosis and mitochondrial dysfunction in human hepatoma HepG2 cells. *European journal of pharmacology* 668.1-2 (2011): 15.
KE6⟶ KE7 Caspase activation causes apoptosis	(1) Hernandez-Baltazar, D., et al. Activation of GSK-3β and caspase-3 occurs in nigral dopamine neurons during the development of apoptosis activated by a striatal injection of 6-hydroxydopamine. *PloS one* 8.8 (2013): e70951. (2) Li, F., et al. Dysregulated expression of secretogranin III is involved in neurotoxin-induced dopaminergic neuron apoptosis. *Journal of neuroscience research* 90.12 (2012): 2237. (3) Mei, J.-M., et al. Effects of CDNF on 6-OHDA-induced apoptosis in PC12 cells via modulation of Bcl-2/Bax and caspase-3 activation. *Neurological Sciences* 35.8 (2014): 1275. (4) Li, D.-W., et al. Guanosine exerts neuroprotective effects by reversing mitochondrial dysfunction in a cellular model of Parkinson's disease. *International journal of molecular medicine* 34.5 (2014): 1358. (5) Naoi, M., et al. Glutathione redox status in mitochondria and cytoplasm differentially and sequentially activates apoptosis cascade in dopamine-melanin-treated SH-SY5Y cells. *Neuroscience letters* 465.2 (2009): 118.

Table 2: Continued.

Pathway 1B	
KE1 ⟶ KE8 Oxidative stress causes UPS dysfunction	(1) Launay, N., et al. Oxidative stress regulates the ubiquitin–proteasome system and immunoproteasome functioning in a mouse model of X-adrenoleukodystrophy. *Brain* 136.3 (2013): 891. (2) Sun, F., et al. Environmental neurotoxic chemicals-induced ubiquitin proteasome system dysfunction in the pathogenesis and progression of Parkinson's disease. *Pharmacology & therapeutics* 114.3 (2007): 327. (3) Chondrogianni, N., et al. Protein damage, repair and proteolysis. *Molecular aspects of medicine* 35 (2014): 1. (4) Bendotti, C., et al. Dysfunction of constitutive and inducible ubiquitin-proteasome system in amyotrophic lateral sclerosis: implication for protein aggregation and immune response. *Progress in neurobiology* 97.2 (2012): 101. (5) Hauser, D.N., et al. Mitochondrial dysfunction and oxidative stress in Parkinson's disease and monogenic parkinsonism. *Neurobiology of disease* 51 (2013): 35. (6) Dias, V., et al. The role of oxidative stress in Parkinson's disease. *Journal of Parkinson's disease* 3.4 (2013): 461.
KE8⟶ KE9 UPS dysfunction causes protein aggregation	(1) Bendotti, C., et al. Dysfunction of constitutive and inducible ubiquitin-proteasome system in amyotrophic lateral sclerosis: implication for protein aggregation and immune response. *Progress in neurobiology* 97.2 (2012): 101. (2) Ebrahimi-Fakhari, D., et al. Protein degradation pathways in Parkinson's disease: curse or blessing. *Acta neuropathologica* 124.2 (2012): 153. (3) Riederer, B.M., et al. The role of the ubiquitin proteasome system in Alzheimer's disease. *Experimental Biology and Medicine* 236.3 (2011): 268. (4) Wu, J., et al. Effects of titanium dioxide nanoparticles on α-synuclein aggregation and the ubiquitin-proteasome system in dopaminergic neurons. *Artificial cells, nanomedicine, and biotechnology* 44.2 (2016): 690. (5) Chu, Y., et al. Alterations in lysosomal and proteasomal markers in Parkinson's disease: relationship to alpha-synuclein inclusions. *Neurobiology of disease* 35.3 (2009): 385. (6) Sass, M.B., et al. A pragmatic approach to biochemical systems theory applied to an α-synuclein-based model of Parkinson's disease. *Journal of neuroscience methods* 178.2 (2009): 366. (7) Sun, F., et al. Environmental neurotoxic chemicals-induced ubiquitin proteasome system dysfunction in the pathogenesis and progression of Parkinson's disease. *Pharmacology & therapeutics* 114.3 (2007): 327.
KE9⟶ KE3 Protein aggregation causes mitochondrial membrane depolarization	(1) Li, L., et al. Human A53T α-synuclein causes reversible deficits in mitochondrial function and dynamics in primary mouse cortical neurons. *PLoS One* 8.12 (2013): e85815. (2) Chen, M., et al. Age-dependent alpha-synuclein accumulation is correlated with elevation of mitochondrial TRPC3 in the brains of monkeys and mice. *Journal of Neural Transmission* 124.4 (2017): 441. (3) Luth, E.S., et al. Soluble, prefibrillar α-synuclein oligomers promote complex I-dependent, Ca2+-induced mitochondrial dysfunction. *Journal of Biological Chemistry* 289.31 (2014): 21490. (4) Sarafian, T.A., et al. Impairment of mitochondria in adult mouse brain overexpressing predominantly full-length, N-terminally acetylated human α-synuclein. *PloS one* 8.5 (2013): e63557. (5) He, Q., et al. Alpha-synuclein aggregation is involved in the toxicity induced by ferric iron to SK-N-SH neuroblastoma cells. *Journal of neural transmission* 118.3 (2011): 397. (6) Ebrahim, A.S., et al. Reduced expression of peroxisome-proliferator activated receptor gamma coactivator-1α enhances α-synuclein oligomerization and down regulates AKT/GSK3β signaling pathway in human neuronal cells that inducibly express α-synuclein. *Neuroscience letters* 473.2 (2010): 120. (7) Cleeter, M.W.J., et al. Glucocerebrosidase inhibition causes mitochondrial dysfunction and free radical damage. *Neurochemistry international* 62.1 (2013): 1.

Table 2: Continued.

Pathway 2	
MIE ⟶ KE10 Increased ROS causes glutathione depletion	(1) Mailloux, R., et al. Unearthing the secrets of mitochondrial ROS and glutathione in bioenergetics. *Trends in biochemical sciences* 38.12 (2013): 592. (2) Ross, E.K., et al. Immunocal® and preservation of glutathione as a novel neuroprotective strategy for degenerative disorders of the nervous system. *Recent patents on CNS drug discovery* 7.3 (2012): 230. (3) Meyer, A.J. The integration of glutathione homeostasis and redox signaling. *Journal of plant physiology* 165.13 (2008): 1390. (4) Hadi, T., et al. Glutathione prevents preterm parturition and fetal death by targeting macrophage-induced reactive oxygen species production in the myometrium. *The FASEB Journal* 29.6 (2015): 2653. (5) You, B.R., et al. Reactive oxygen species, glutathione, and thioredoxin influence suberoyl bishydroxamic acid-induced apoptosis in A549 lung cancer cells. *Tumor Biology* 36.5 (2015): 3429. (6) Timme-Laragy, A.R., et al. Glutathione redox dynamics and expression of glutathione-related genes in the developing embryo. *Free Radical Biology and Medicine* 65 (2013): 89. (7) You, B.R., et al. Gallic acid-induced lung cancer cell death is accompanied by ROS increase and glutathione depletion. *Molecular and cellular biochemistry* 357.1-2 (2011): 295. (8) Dunning, S., et al. Glutathione and antioxidant enzymes serve complementary roles in protecting activated hepatic stellate cells against hydrogen peroxide-induced cell death. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* 1832.12 (2013): 2027. (9) You, B.R., et al. Arsenic trioxide induces human pulmonary fibroblast cell death via increasing ROS levels and GSH depletion. *Oncology reports* 28.2 (2012): 749. (10) Quintana-Cabrera, R., et al. Glutathione and γ-glutamylcysteine in the antioxidant and survival functions of mitochondria. (2013): 106. (11) Thushara, R.M., et al. Sesamol induces apoptosis in human platelets via reactive oxygen species-mediated mitochondrial damage. *Biochimie* 95.11 (2013): 2060.
Pathway 2A	
KE10 ⟶ KE1 Glutathione depletion causes oxidative stress	(1) Vaziri, N.D., et al. Induction of oxidative stress by glutathione depletion causes severe hypertension in normal rats. *Hypertension* 36.1 (2000): 142. (2) Schulz, J.B., et al. Glutathione, oxidative stress and neurodegeneration. *The FEBS Journal* 267.16 (2000): 4904. (3) Shang, Y., et al. Downregulation of glutathione biosynthesis contributes to oxidative stress and liver dysfunction in acute kidney injury. *Oxidative medicine and cellular longevity* 2016 (2016). (4) Trocino, R.A., et al. Significance of glutathione depletion and oxidative stress in early embryogenesis in glucose-induced rat embryo culture. *Diabetes* 44.8 (1995): 992. (5) Zlatković, J., et al. Chronic administration of fluoxetine or clozapine induces oxidative stress in rat liver: a histopathological study. *European Journal of Pharmaceutical Sciences* 59 (2014): 20. (6) Iguchi, Y., et al. Oxidative stress induced by glutathione depletion reproduces pathological modifications of TDP-43 linked to TDP-43 proteinopathies. *Neurobiology of disease* 45.3 (2012): 862. (7) Jung, C.L., et al. Synergistic activation of the Nrf2-signaling pathway by glyceollins under oxidative stress induced by glutathione depletion. *Journal of agricultural and food chemistry* 61.17 (2013): 4072. (8) Won, S.J., et al. Assessment at the single-cell level identifies neuronal glutathione depletion as both a cause and effect of ischemia-reperfusion oxidative stress. *Journal of Neuroscience* 35.18 (2015): 7143. (9) De Vos, C.H.R., et al. Glutathione depletion due to copper-induced phytochelatin synthesis causes oxidative stress in Silene cucubalus. *Plant physiology* 98.3 (1992): 853.

Table 2: Continued.

Pathway 2B	
KE10 ⟶KE11 Glutathione depletion ⟶ Calcium dysregulation	(1) Övey, I.S., et al. Homocysteine and cytosolic GSH depletion induce apoptosis and oxidative toxicity through cytosolic calcium overload in the hippocampus of aged mice: involvement of TRPM2 and TRPV1 channels. *Neuroscience* 284 (2015): 225. (2) Frosali, S., et al. Role of intracellular calcium and S-glutathionylation in cell death induced by a mixture of isothiazolinones in HL60 cells. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research* 1793.3 (2009): 572. (3) Orihuela, D., et al. Aluminium-induced impairment of transcellular calcium absorption in the small intestine: calcium uptake and glutathione influence. *Journal of inorganic biochemistry* 99.9 (2005): 1879. (4) Macho, A., et al. Glutathione depletion is an early and calcium elevation is a late event of thymocyte apoptosis. *The Journal of Immunology* 158.10 (1997): 4612. (5) Grewal, K.K., et al. Bromobenzene and furosemide hepatotoxicity: alterations in glutathione, protein thiols, and calcium. *Canadian journal of physiology and pharmacology* 74.3 (1996): 257. (6) Singh, B.K., et al. Nimesulide aggravates redox imbalance and calcium dependent mitochondrial permeability transition leading to dysfunction in vitro. *Toxicology* 275.1-3 (2010): 1. (7) Vendemiale, G., et al. Effect of acetaminophen administration on hepatic glutathione compartmentation and mitochondrial energy metabolism in the rat. *Biochemical pharmacology* 52.8 (1996): 1147. (8) Marchionatti, A.M., et al. Mitochondrial dysfunction is responsible for the intestinal calcium absorption inhibition induced by menadione. *Biochimica et Biophysica Acta (BBA)-General Subjects* 1780.2 (2008): 101. (9) Özgül, C., et al. TRPM2 channel protective properties of N-acetylcysteine on cytosolic glutathione depletion dependent oxidative stress and Ca2+ influx in rat dorsal root ganglion. *Physiology & behavior* 106.2 (2012): 122. (10) Yang, B.-C., et al. Crotonaldehyde induces apoptosis in alveolar macrophages through intracellular calcium, mitochondria and p53 signaling pathways. *The Journal of toxicological sciences* 38.2 (2013): 225. (11) Övey, I.S., et al. Homocysteine and cytosolic GSH depletion induce apoptosis and oxidative toxicity through cytosolic calcium overload in the hippocampus of aged mice: involvement of TRPM2 and TRPV1 channels. *Neuroscience* 284 (2015): 225. (12) Thushara, R.M., et al. Sesamol induces apoptosis in human platelets via reactive oxygen species-mediated mitochondrial damage. *Biochimie* 95.11 (2013): 2060. (13) Nazıroğlu, M., et al. Neuroprotection induced by N-acetylcysteine against cytosolic glutathione depletion-induced Ca2+ influx in dorsal root ganglion neurons of mice: role of TRPV1 channels. *Neuroscience* 242 (2013): 151.

Table 2: Continued.

KE11⟶ KE4 Calcium dysregulation causes opening of mPTP	(1) Lu, C., et al. Role of calcium and cyclophilin D in the regulation of mitochondrial permeabilization induced by glutathione depletion. *Biochemical and biophysical research communications* 363.3 (2007): 572. (2) Baumgartner, H.K., et al. Calcium elevation in mitochondria is the main Ca2+ requirement for mitochondrial permeability transition pore (mPTP) opening. *Journal of Biological Chemistry* 284.31 (2009): 20796. (3) Thushara, R.M., et al. Sesamol induces apoptosis in human platelets via reactive oxygen species-mediated mitochondrial damage. *Biochimie* 95.11 (2013): 2060. (4) Zhang, S., et al. Critical roles of intracellular thiols and calcium in parthenolide-induced apoptosis in human colorectal cancer cells. *Cancer letters* 208.2 (2004): 143. (5) Bernardi, P. Mitochondria in muscle cell death. *The Italian Journal of Neurological Sciences* 20.6 (1999): 395. (6) Yang, B.-C., et al. Crotonaldehyde induces apoptosis in alveolar macrophages through intracellular calcium, mitochondria and p53 signaling pathways. *The Journal of toxicological sciences* 38.2 (2013): 225. (7) Berson, A., et al. The anti-inflammatory drug, nimesulide (4-nitro-2-phenoxymethane-sulfoanilide), uncouples mitochondria and induces mitochondrial permeability transition in human hepatoma cells: protection by albumin. *Journal of Pharmacology and Experimental Therapeutics* 318.1 (2006): 444. (8) Lee, G.-H., et al. Bax inhibitor-1-mediated inhibition of mitochondrial Ca 2+ intake regulates mitochondrial permeability transition pore opening and cell death. *Scientific reports* 4 (2014): 5194. (9) Barsukova, A., et al. Activation of the mitochondrial permeability transition pore modulates Ca2+ responses to physiological stimuli in adult neurons. *European Journal of Neuroscience* 33.5 (2011): 831. (10) Moon, S.H., et al. Genetic ablation of calcium-independent phospholipase A2γ (iPLA2γ) attenuates calcium-induced opening of the mitochondrial permeability transition pore and resultant cytochrome c release. *Journal of Biological Chemistry* 287.35 (2012): 29837. (11) Yamamoto, T., et al. The mechanisms of the release of cytochrome C from mitochondria revealed by proteomics analysis. *Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan* 132.10 (2012): 1099. (12) Rasola, A., et al. The mitochondrial permeability transition pore and its involvement in cell death and in disease pathogenesis. *Apoptosis* 12.5 (2007): 815. (13) De la Fuente, S., et al. The Spatial Distribution of the Na+/Ca2+ Exchanger in Cardiac Mitochondria Enhances the Efficincy of the Mitochondrial Ca2+ Signal Generation. *Biophysical Journal* 114.3 (2018): 659a. (14) Kannurpatti, S.S., et al. Calcium sequestering ability of mitochondria modulates influx of calcium through glutamate receptor channel. *Neurochemical research* 25.12 (2000): 1527. (15) Rueda, C.B., et al. Glutamate excitotoxicity and Ca2+-regulation of respiration: Role of the Ca2+ activated mitochondrial transporters (CaMCs). *Biochimica et Biophysica Acta (BBA)-Bioenergetics* 1857.8 (2016): 1158. (16) Baines, C.P., et al. Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* 434.7033 (2005): 658. (17) Basso, E., et al. Properties of the permeability transition pore in mitochondria devoid of Cyclophilin D. *Journal of Biological Chemistry* 280.19 (2005): 18558.

Table 2: Continued.

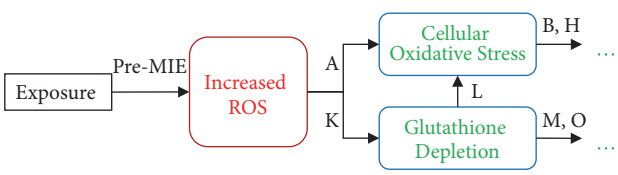
Pathway 2C	
KE10⟶ KE12 Glutathione depletion causes ATP depletion	(1) Almeida, A., et al. Glutamate neurotoxicity is associated with nitric oxide-mediated mitochondrial dysfunction and glutathione depletion. *Brain research* 790.1-2 (1998): 209. (2) Vesce, S., et al. Acute glutathione depletion restricts mitochondrial ATP export in cerebellar granule neurons. *Journal of Biological Chemistry* 280.46 (2005): 38720. (3) Schütt, F., et al. Moderately reduced ATP levels promote oxidative stress and debilitate autophagic and phagocytic capacities in human RPE cells. *Investigative ophthalmology & visual science* 53.9 (2012): 5354. (4) Huang, J., et al. Cellular responses of cultured cerebellar astrocytes to ethacrynic acid-induced perturbation of subcellular glutathione homeostasis. *Brain research* 711.1-2 (1996): 184. (5) Zeevalk, G.D., et al. Energy Stress-Induced Dopamine Loss in Glutathione Peroxidase-Overexpressing Transgenic Mice and in Glutathione-Depleted Mesencephalic Cultures. *Journal of neurochemistry* 68.1 (1997): 426. (6) Den Boer, P.J., et al. Effect of glutathione depletion on the cytotoxicity of xenobiotics and induction of single-strand DNA breaks by ionizing radiation in isolated hamster round spermatids. *Journal of reproduction and fertility* 88.1 (1990): 259. (7) Vesce, S., et al. Acute glutathione depletion restricts mitochondrial ATP export in cerebellar granule neurons. *Journal of Biological Chemistry* 280.46 (2005): 38720. (8) Navarini, A.L.F., et al. Hydroxychalcones induce apoptosis in B16-F10 melanoma cells via GSH and ATP depletion. *European journal of medicinal chemistry* 44.4 (2009): 1630. (9) Locatelli, C., et al. Gallic acid ester derivatives induce apoptosis and cell adhesion inhibition in melanoma cells: the relationship between free radical generation, glutathione depletion and cell death. *Chemico-biological interactions* 181.2 (2009): 175. (10) Raza, H., et al. Acetylsalicylic acid-induced oxidative stress, cell cycle arrest, apoptosis and mitochondrial dysfunction in human hepatoma HepG2 cells. *European journal of pharmacology* 668.1-2 (2011): 15. (11) Mithöfer, K., et al. Mitochondrial poisons cause depletion of reduced glutathione in isolated hepatocytes. *Archives of biochemistry and biophysics* 295.1 (1992): 132.

Table 2: Continued.

KE12⟶ KE11 ATP depletion causes calcium dysregulation	(1) Bruce, J.I.E. Plasma membrane calcium pump regulation by metabolic stress. *World journal of biological chemistry* 1.7 (2010): 221. (2) Spivey, J.R., et al. Glycochenodeoxycholate-induced lethal hepatocellular injury in rat hepatocytes. Role of ATP depletion and cytosolic free calcium. *The Journal of clinical investigation* 92.1 (1993): 17. (3) Akopova, O.V., et al. The effect of ATP-dependent K (+)-channel opener on the functional state and the opening of cyclosporine-sensitive pore in rat liver mitochondria. *Ukrains' kyi biokhimichnyi zhurnal (1999)* 85.3 (2013): 38. (4) Zhang, R., et al. Involvement of calcium, reactive oxygen species, and ATP in hexavalent chromium-induced damage in red blood cells. *Cellular Physiology and Biochemistry* 34.5 (2014): 1780. (5) James, A.D., et al. Glycolytic ATP fuels the plasma membrane calcium pump critical for pancreatic cancer cell survival. *Journal of Biological Chemistry* 288.50 (2013): 36007. (6) James, A.D., et al. The plasma membrane calcium pump in pancreatic cancer cells exhibiting the Warburg effect relies on glycolytic ATP. *Journal of Biological Chemistry* 290.41 (2015): 24760. (7) Cejka, J.-C., et al. Activation of calcium influx by ATP and store depletion in primary cultures of renal proximal cells. *Pflügers Archiv* 427.1-2 (1994): 33. (8) Chao, C.-C., et al. Ca2+ store depletion and endoplasmic reticulum stress are involved in P2X7 receptor-mediated neurotoxicity in differentiated NG108-15 cells. *Journal of cellular biochemistry* 113.4 (2012): 1377. (9) Mattson, M.P., et al. Energetics and oxidative stress in synaptic plasticity and neurodegenerative disorders. *Neuromolecular medicine* 2.2 (2002): 215. (10) Bernardi, P. Mitochondria in muscle cell death. *The Italian Journal of Neurological Sciences* 20.6 (1999): 395. (11) Berson, A., et al. The anti-inflammatory drug, nimesulide (4-nitro-2-phenoxymethane-sulfoanilide), uncouples mitochondria and induces mitochondrial permeability transition in human hepatoma cells: protection by albumin. *Journal of Pharmacology and Experimental Therapeutics* 318.1 (2006): 444.
Adverse Outcomes	
KE7⟶ AO1 Apoptosis causes mitochondrial disease	(1) Du, Ya., et al. Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. *Proceedings of the National Academy of Sciences* 98.25 (2001): 14669. (2) Chung, K.K.K., et al. New insights into Parkinson's disease. *Journal of neurology* 250.3 (2003): iii15. (3) Thomas, K.J., et al. The role of PTEN-induced kinase 1 in mitochondrial dysfunction and dynamics. *The international journal of biochemistry & cell biology* 41.10 (2009): 2025. (4) Nordström, U., et al. Progressive nigrostriatal terminal dysfunction and degeneration in the engrailed1 heterozygous mouse model of Parkinson's disease. *Neurobiology of disease* 73 (2015): 70. (5) Van der Perren, A., et al. Longitudinal follow-up and characterization of a robust rat model for Parkinson's disease based on overexpression of alpha-synuclein with adeno-associated viral vectors. *Neurobiology of aging* 36.3 (2015): 1543. (6) Ares-Santos, S., et al. Methamphetamine causes degeneration of dopamine cell bodies and terminals of the nigrostriatal pathway evidenced by silver staining. *Neuropsychopharmacology* 39.5 (2014): 1066. (7) Gantz, S.C., et al. Loss of Mecp2 in substantia nigra dopamine neurons compromises the nigrostriatal pathway. *Journal of Neuroscience* 31.35 (2011): 12629. (8) Panayotis, N., et al. Morphological and functional alterations in the substantia nigra pars compacta of the Mecp2-null mouse. *Neurobiology of disease* 41.2 (2011): 385. (9) Matsuura, K., et al. Cyclosporin A attenuates degeneration of dopaminergic neurons induced by 6-hydroxydopamine in the mouse brain. *Brain research* 733.1 (1996): 101. (10) Walters, T.L., et al. Diethyldithiocarbamate causes nigral cell loss and dopamine depletion with nontoxic doses of MPTP. *Experimental neurology* 156.1 (1999): 62. (11) Raza, H., et al. Acetylsalicylic acid-induced oxidative stress, cell cycle arrest, apoptosis and mitochondrial dysfunction in human hepatoma HepG2 cells. *European journal of pharmacology* 668.1-2 (2011): 15.

Table 2: Continued.

AO1 ⟶ AO2 Mitochondrial disease causes symptoms of organ system failure	(1) Crouser, E.D. Mitochondrial dysfunction in septic shock and multiple organ dysfunction syndrome. *Mitochondrion* 4.5 (2004): 729. (2) Ares-Santos, S., et al. Methamphetamine causes degeneration of dopamine cell bodies and terminals of the nigrostriatal pathway evidenced by silver staining. *Neuropsychopharmacology* 39.5 (2014): 1066. (3) Colebrooke, R.E., et al. Age-related decline in striatal dopamine content and motor performance occurs in the absence of nigral cell loss in a genetic mouse model of Parkinson's disease. *European Journal of Neuroscience* 24.9 (2006): 2622. (4) Panayotis, N., et al. Morphological and functional alterations in the substantia nigra pars compacta of the Mecp2-null mouse. *Neurobiology of disease* 41.2 (2011): 385. (5) Willard, A.M., et al. Differential degradation of motor deficits during gradual dopamine depletion with 6-hydroxydopamine in mice. *Neuroscience* 301 (2015): 254. (6) Heuer, A., et al. Dopamine-rich grafts alleviate deficits in contralateral response space induced by extensive dopamine depletion in rats. *Experimental neurology* 247 (2013): 485. (7) Plowman, E.K., et al. Differential sensitivity of cranial and limb motor function to nigrostriatal dopamine depletion. *Behavioural brain research* 237 (2013): 157. (8) Plowman, E.K., et al. Striatal dopamine depletion induces forelimb motor impairments and disrupts forelimb movement representations within the motor cortex. *Journal of Parkinson's disease* 1.1 (2011): 93. (9) Christopher, L., et al. Combined insular and striatal dopamine dysfunction are associated with executive deficits in Parkinson's disease with mild cognitive impairment. *Brain* 137.2 (2013): 565. (10) Plowman, E.K., et al. Behavioral and neurophysiological correlates of striatal dopamine depletion: A rodent model of Parkinson's disease. *Journal of communication disorders* 44.5 (2011): 549. (11) Charlton, C.G., et al. Striatal dopamine depletion, tremors, and hypokinesia following the intracranial injection of S-adenosylmethionine. *Molecular and chemical neuropathology* 26.3 (1995): 269.



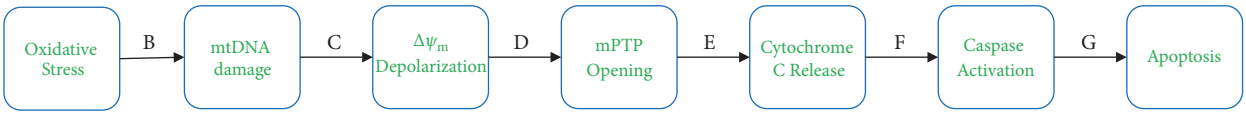
Weight of Evidence

KER	Insufficient	Weak	Moderate	Strong
Pre-MIE				✓
A				✓
K				✓
L				✓

Plausibility

- A significant amount of research shows that chloroform, chloroacetic acid, and chlorophenol exposure to mammalian cells cause an increase in reactive oxygen species (ROS) generation *(Pre-MIE)*.
- Increased ROS concentration caused by exposure to these organohalogens has been found to cause oxidative stress *(A)* and glutathione depletion *(K)*.
- Glutathione is an essential antioxidant and is one of the cell's main defenses against reactive oxygen species. When cells are exposed to a toxic amount of these organohalogens, the amount of ROS generated exceeds the amount of mitochondrial glutathione being produced, and glutathione stores become depleted.
- Glutathione depletion further increases cellular oxidative stress *(L)* because the cell lacks one of its main antioxidants.
- Glutathione depletion is a key event in the apoptotic cascade.

Figure 4: Exposure to MIE relationship is strong. A qualitative assessment of Key Event Relationships (KERs) in the AOP triggered by the generation of reactive oxygen species (MIE) and resulting in mitochondrial disease (AO) via KERs A, K, and L.



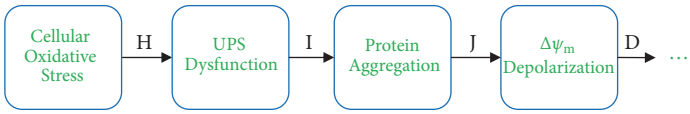
Weight of Evidence

KER	Insufficient	Weak	Moderate	Strong
B				✓
C		✓		
D			✓	
E			✓	
F			✓	
G			✓	

Plausibility

- Oxidative stress causes strand breaks in mitochondrial DNA (mtDNA) and loss of its supercoiled structure, leading to genetic mutations *(B)*.
- mtDNA is recognized as a major cause of cellular apoptosis, but the mechanisms of this are still not clear. Based on our research, we believe that the above-mentioned changes in mtDNA directly contribute to depolarization of the mitochondrial membrane *(C)*. Unfortunately, there is not an abundant amount of literature on this, so it this KER is labeled as "weak". This mechanism should be studied further.
- Depolarization of the mitochondrial membrane causes opening of the mitochondrial permeability transition pore (mPTP) *(D)*.
- mPTP opening allows rapid release of cytochrome c into the cell *(E)*.
- Upon release from the mitochondria, cytochrome c activates caspases *(F)*, which trigger apoptosis *(G)*.

Figure 5: Analyses of Pathway IA. A qualitative assessments of Key Event Relationships (KERs) in the AOP triggered by the generation of reactive oxygen species (MIE) and resulting in mitochondrial disease (AO) via KERs B, C, D, E, F, and G.



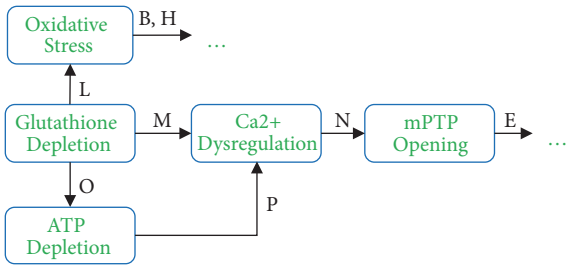
Weight of Evidence

KER	Insufficient	Weak	Moderate	Strong
H			✓	
I				✓
J			✓	

Plausibility

- Increased oxidative stress can disrupt the normal function of the ubiquitin proteasome system (UPS).
- UPS is responsible for clearing out aggregated proteins.
- UPS dysfunction promotes aggregated protein formation *(I)*.
- The accumulation of protein aggregates is associated with decreased mitochondrial membrane potential

Figure 6: Analyses of Pathway IB. A qualitative assessments of Key Event Relationships (KERs) in the AOP triggered by the generation of reactive oxygen species (MIE) and resulting in mitochondrial disease (AO) via KERs H, I, and J.



Weight of Evidence

KER	Insufficient	Weak	Moderate	Strong
L				✓
M				✓
N				✓
O				✓
P				✓

Plausibility

- The depletion of glutathione leads to three key events, including oxidative stress *(L)*, calcium dysregulation *(M)*, and ATP depletion *(O)*.
- Both glutathione and ATP depletion lead to calcium dysregulation within the cell *(M, P)*.
- Glutathione depletion disrupts calcium homeostasis by causing a large influx of Ca^{2+} into the cytosol.
- ATP depletion contributes to calcium dysregulation in the same manner (as glutathione depletion).
- In an attempt to mediate the high levels of Ca^{2+} in the cytosol, the mitochondria sequesters cytosolic calcium into the mitochondrial matrix, leading to an overload of calcium in the mitochondria.
- Increased mitochondrial Ca^{2+} triggers opening of the Ca^{2+}-mediated mitochondrial permeability transition pore (mPTP) *(N)*.

Figure 7: Analyses of Pathway II. A qualitative assessments of Key Event Relationships (KERs) in the AOP triggered by the generation of reactive oxygen species (MIE) and resulting in mitochondrial disease (AO) via KERs L, M, N, O, and P.

lists the physicochemical characteristics of three notable organohalogens (i.e., chloroform, trichloroacetic acid, and trichlorophenol) that are believed to inhibit the normal function of mitochondria in mammalian cells.

There is extensive indirect evidence of chloroform acting as an inhibitor of the electron transport chain through the generation of ROS, induction of oxidative stress conditions, and depletion of glutathione and ATP [68, 69]. Exposure to chloroform induces a dose-dependent cytotoxic response. It is classified as a Group 2B carcinogen by the IARC, however its propensity to induce tumors in humans is limited. LD_{50} values assessed from oral exposure to chloroform vary in rodent studies; values range 446 mg/kg for 14-day-olds male rats to 2,180 mg/kg for adult rats [51, 52]. But the mechanism of action at low-dose exposures is not yet established. Data suggest that chloroform metabolites produce DNA mutations [53, 54]. Chloroform-induced cell death is observed through the biochemical hallmarks of apoptosis, cytochrome c release, and activation of caspases 3 and 9. Cytochrome c is released into the cytosol in a time-dependent manner. The longer cells are exposed to organohalogens, the more cytochrome c is released.

Chloroacetic acid also interferes with the mitochondria. Unlike chloroform, it is not classified as a carcinogen. The metabolites of chloroacetic acid, including glycolic acid and oxalate, do generate ROS within the cytosol and quickly deplete glutathione supplies. The LD_{50} value of chloroacetic acid/trichloroacetic acid is reported to be 425 mg/kg (rats, oral) [55]. Chlorophenol acts in a similar manner but at lower doses (LD_{50} of 670 mg/kg (rats, oral)), making it more cytotoxic than chloroform (695 mg/kg), but less toxic than chloroacetic acid (425 mg/kg) [56–59]. Chloroacetic acid may be less cytotoxic because of its high-water solubility (greater than 10,000 g/L at 20°C). It is able to metabolize quickly through Phase 1 and Phase 2 processes as compared to the less water-soluble compounds (trichloromethane (8.09 g/L) and trichlorophenol (20.0 g/L)). Trichlorophenol may be more cytotoxic because of its aromatic structure. Aromatic compounds are generally metabolized by P450 enzymes and form epoxides, which cause DNA damage (mitochondrial or nuclear).

3.1.2. Graphical Representation and Plausibility of the KERs. Many of the individual KERs within this developed AOP are strong. Figure 3 presents the overall graphical representation of organohalogen exposure to mitochondrial disease with each pathway's biological plausibility detailed in subsequent figures. Refer to Table 2 for the specific references from the scientific literature that support each of these KERs.

The electron transport chain, located on the inner membrane of the mitochondria, is the site of oxidative phosphorylation. When mammalian cells are exposed to organohalogens, their electron transport chain produces an increased amount of reactive oxygen species (ROS), inducing oxidative stress conditions within the cell (A). In an attempt to mediate oxidative stress, glutathione, a powerful antioxidant, is put to work. Eventually, ROS is produced faster than the cell produces antioxidants and glutathione stores are depleted (K), allowing the oxidative stress conditions to persist (L).

Because mitochondrial DNA is housed so close to the electron transport chain, it is exposed to high amounts of ROS, which causes strand breakage and loss of its tertiary structure (B). These changes affect mtDNA translation, eventually leading to depolarization of the mitochondrial membrane (C). Depolarization of the mitochondrial membrane contributes to opening of the mitochondrial permeability

transition pore, or mPTP (D). When the mitochondrial membrane opens, cytochrome c is released into the cytosol of the cell (E). Cytochrome c activates caspases (F), which trigger the apoptotic cascade (G).

In addition to mitochondrial DNA damage, oxidative stress conditions also inhibit the function of the ubiquitin proteasome system (H), or UPS, which functions to clear out aggregated proteins within the cell. UPS dysfunction leads to a buildup of aggregated proteins within the cell (I). These aggregated proteins also contribute to depolarization of the mitochondrial membrane (J), which then triggers opening of the mPTP, cytochrome c release, caspase activation, and apoptosis.

Glutathione depletion caused by increased ROS production contributes to sustained oxidative stress conditions (L) and inhibits the production of ATP (O) in complex I of the mitochondrial electron transport chain. Both glutathione depletion and ATP depletion cause an interruption of calcium homeostasis within the cell (M, P). These conditions cause a large influx of calcium into the cell, which is then transported into the mitochondria in an attempt to regulate cellular calcium levels. This causes an overload of calcium in the mitochondria, which triggers opening of the calcium-mediated mPTP (N). Opening of the mPTP triggers cytochrome c release, caspase activation, and apoptosis, as discussed above.

Caspase activation causes two notable adverse biochemical reactions. The first is apoptosis, which occurs at the cellular level (G). The second is mitochondrial disease, which occurs at the tissue level. Apoptosis in cells with high mitochondrial content also contributes to the onset of mitochondrial disease. Mitochondria are responsible for providing cells with the energy needed to function normally. When mitochondrial disease is present, the cells are unable to function as efficiently, and therefore the organs are unable to function efficiently. This leads to symptoms of organ system failure or dysfunction. Apoptosis in cells with high mitochondria content can also lead to symptoms of organ dysfunction. Symptoms vary, depending on which organ(s) are affected by the mitochondrial dysfunction as well as which part of the organ is affected. For example, mitochondrial disease affecting the nigrostriatal pathway in the brain can cause motor deficits as manifested by Parkinson's disease [70]. Meanwhile, mitochondrial disease of the heart muscle can cause edema, shortness of breath, and arrhythmias, as manifested by mitochondrial cardiomyopathy [71].

It is important to note that many of the KEs presented in these pathways contain positive feedback loops. For example, calcium dysregulation, glutathione depletion, and ATP depletion all contribute to a further increase in ROS generation. These feedback loops were omitted from the figures for the sake of simplicity, but it is an important to recognize that these synergistic relationships do exist.

3.2. Applicability of the AOP. In terms of taxonomic applicability, this AOP describes the toxic mechanisms of organohalogens on mitochondrial electron transport chain and is therefore relevant to any organism that utilizes mitochondria for energy production. Mitochondrial disease can be caused by mitochondria respiratory deficiencies, which are often present at birth or in early childhood and typically result in progressive muscular and neurological degenerative disorders. Adult onset of mitochondria respiratory defects is increasingly common, thus increasing the applicability of organohalogens-to-mitochondrial disease AOP to multiple stages of life. Mitochondria serve several roles in maintaining homeostasis and these roles evolve from fertilized egg to old age. Although this AOP focuses on organohalogen exposure, this pathway can be adapted to fit a wide range of environmental triggers of mitochondrial disease.

4. Discussion

An AOP was created to best characterize the pathway-based analysis of organohalogen exposure that results in dysfunction of the mitochondrial electron transport chain in humans (Figure 1). For the purposes of this AOP, more emphasis was placed on the front-end of the AOP (MIE and the KEs) than the back-end (the AO). Mitochondrial disease can present itself in various physiological or toxicological manifestations, which makes it difficult to pinpoint one specific disease of interest. Instead, we provide evidence of the MIE responsible for initiating irreversible damage to mitochondria, which causes cell death and progression towards a diagnosable mitochondrial disease.

There is a suite of exogenous substances that have be shown to trigger changes in mitochondrial flux. While organohalogen compounds are highly cited as generators of ROS in cell and tissue systems, other materials, such as exhaust fumes, pesticides, tobacco products, smoke, drugs, or metals have been shown to increase oxidative stress in biological tests systems [72–74]. Furthermore, intangible stressors, such as radiation, heat, or ultraviolet light exposure, induce similar oxidative stress endpoints [75–78]. Most recently, exposure to engineered nanomaterials has been consistently linked to ROS generation in both cell-based and cell-free test systems which has been implicated as the main source of inflammatory responses in rodent studies [79–81].

Relating individual components of this AOP to other chemicals, particles, and fibers is possible. Inter-relationships between (or the ability to read-across) multiple test substances are dependent upon the level of biological organization. For example, the properties among classes of test substances vary greatly; i.e., chemicals are generally in a liquid form where particles and fibers are in a solid form. Liquids and solids have different physicochemical characteristics and hence the molecular initiating event is dependent upon the immediate chemical reaction between the substance of interest and subcellular entities; the physical (shape or size) and chemical (solubility or composition) characteristics often dictate the biochemical effects. Therefore, there are limitations in establishing interrelationships among different materials. However, as the AOP moves downstream from MIE to higher levels of biological organization, the dependency on physicochemical properties of the test system becomes less pronounced. Key events, key event relationships, and adverse outcomes at the tissue, organ, and individual levels

are increasingly independent of material properties. Therefore, the ability to define interrelationships among classes of materials is possible.

The MIE is considered the "first domino" of the pathway, meaning that once the MIE occurs, reversible or irreversible damage will continuously cause KEs until the AO is achieved. Without the MIE, the progression of the AOP is halted and the KEs will not occur [47, 49, 50]. With regards to this AOP, the necessary MIE is the increase in ROS species. We have identified two main pathways that contribute to the development of mitochondrial disease from organohalogen exposure. These pathways diverge from the MIE and converge onto a single KE: opening of the mitochondrial permeability transition pore (mPTP).

Pathway 1 follows the direct effects of oxidative stress conditions caused by increased ROS generation. There are two main effects of oxidative stress identified in this pathway. The first is mitochondrial DNA (mtDNA) damage (Pathway 1A). Because ROS damages protein structures, the free radicals cause mtDNA strand breaks, loss of supercoiled structure, and interrupt mtDNA translation [82–84]. The second is dysfunction of the ubiquitin-proteasome system, which causes protein aggregation (Pathway 1B) [85, 86]. Both mtDNA damage and protein aggregation contribute to depolarization of the mitochondrial membrane, which in turn causes opening of the mPTP [87, 88].

Pathway 2 follows the direct effects of glutathione depletion caused by increased ROS concentration [89]. We have identified three effects of glutathione depletion. The first is that, because glutathione is one of the cell's main antioxidants, its depletion contributes to oxidative stress conditions (Pathway 2A) [90]. Glutathione depletion also causes a dysregulation in calcium homeostasis (Pathway 2B) [91]. When glutathione stores are depleted, a large amount of calcium is sent into the cell, which is then sequestered into the mitochondria and causes calcium overload [92]. This calcium overload within the mitochondria triggers opening of the Ca^{2+}-mediated mPTP [93]. ROS generation and glutathione depletion perturb the mitochondrial electron transport chain [94]. As a result, the enzyme is unable to produce the proton gradient needed for ATP synthase to convert ADP to ATP, and ATP levels are depleted (Pathway 2C) [95]. ATP depletion contributes to calcium dysregulation in the same manner as glutathione depletion, as described above [96].

Pathways 1 and 2 converge on a common Key Event: opening of the mPTP. This is a crucial step in the apoptotic pathway, as it allows release of cytochrome c into the cytosol. Cytochrome c then activates caspases, which are responsible for programmed cell death.

The plausibility table included in Figure 5 outlines the research needed to strengthen the weight of evidence for key event C, i.e., mitochondrial DNA damage to depolarization of mitochondrial membrane. A few noteworthy studies have found evidence for this key event relationship [87, 97, 98]. Specifically, however, more evidence is needed to strengthen the KER by demonstrating that oxidative stress causes strand breaks in mitochondrial DNA (mtDNA) and subsequent loss of its supercoiled structure. Loss of structure is believed to lead to genetic mutations. Mitochondrial DNA (mtDNA) is recognized as a major cause of cellular apoptosis, but the exact mechanisms of toxic action are still not clear. Based on our research, we believe that the abovementioned changes in mtDNA directly contribute to depolarization of the mitochondrial membrane. Unfortunately, there is not an abundant amount of literature on event C so it this KER is labeled as "weak". This mechanism should be studied further.

The Adverse Outcomes (AOs) identified in this AOP are mitochondrial disease (at the tissue/organ level) and symptoms of organ system dysfunction (at the individual level). The onset of mitochondrial disease occurs when enough cells in an organ exhibit significant caspase activation and/or induced apoptosis [13, 99]. Caspases cause programmed cell death via apoptosis [100]. After cell death, the tissue and organ to which the deceased cells belong become nonfunctional or nonviable, resulting in tissue and organ system failure. This systemic failure can lead to the typical clinical symptoms associated with mitochondrial disease, such as neurodegeneration, impaired growth, muscle weakness, and developmental delays, before evolving into a diagnosable mitochondrial disease (AO) [101, 102]. In other words, the inhibiting ability of organohalogen compounds has multilevel detrimental effects, including the macromolecular level, cellular/tissue level, organ level, organism level, and population or ecosystem level.

5. Conclusion

Mitochondrial function is an evolving field of study. Its role was previously described in simple terms, such as "mitochondria is the powerhouse of the cell"; however, new research is emerging that enables our understanding of mitochondria-to-disease pathways. With the motivation to reduce the number of animals in experimental designs and to create unprecedented value in the enormous amount of published studies on mitochondrial dysfunction, AOP development has the unique opportunity to serve as a framework for understanding mitochondrial disease. The effect of organohalogen exposure on intracellular respiration and oxidative stress conditions is one example in a long list of possible environmental exposures causing mitochondrial dysfunction. Overall, the biological plausibility of the key events and their relationships is strong and exists in the form of *in vivo* and *in vitro* studies. Although the strength of the biological plausibility of our AOP is strong, the strength of empirical evidence ranges from weak to strong, with the least amount of evidence existing to support the AO.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Brooke McMinn and Alicia L. Duval contributed equally in the preparation of this manuscript.

Acknowledgments

Christie M. Sayes thanks the C. Gus Classcock Jr. Endowed Fund for Excellence in Environmental Sciences and the Department of Environmental Science at Baylor University for generous support of this work.

References

[1] F. Scaglia et al., "Clinical spectrum, morbidity, and mortality in 113 pediatric patients with mitochondrial disease," *Pediatrics*, vol. 114, no. 4, pp. 925–931, 2004.

[2] C. Tzoulis, T. Schwarzlmüller, M. Biermann, K. Haugarvoll, and L. A. Bindoff, "Mitochondrial DNA homeostasis is essential for nigrostriatal integrity," *Mitochondrion*, vol. 28, pp. 33–37, 2016.

[3] S. J. Korsmeyer, "BCL-2 gene family and the regulation of programmed cell death," *Cancer Genetics and Cytogenetics*, vol. 59, 7, no. 2, pp. 1693s–1700s, 1999.

[4] G. Kroemer and J. C. Reed, "Mitochondrial control of cell death," *Nature Medicine*, vol. 6, no. 5, pp. 513–519, 2000.

[5] A. Linkermann, B. R. Stockwell, S. Krautwald, and H.-J. Anders, "Regulated cell death and inflammation: an auto-amplification loop causes organ failure," *Nature Reviews Immunology*, vol. 14, no. 11, pp. 759–767, 2014.

[6] R. S. Hotchkiss, P. E. Swanson, B. D. Freeman et al., "Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction," *Critical Care Medicine*, vol. 27, no. 7, pp. 1230–1251, 1999.

[7] P. M. Kidd, "Alzheimer's disease, amnestic mild cognitive impairment, and age-associated memory impairment: Current understanding and progress toward integrative prevention," *Alternative Medicine Review*, vol. 13, no. 2, pp. 85–115, 2008.

[8] M. E. Risner, A. M. Saunders, J. F. B. Altman et al., "Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease," *The Pharmacogenomics Journal*, vol. 6, no. 4, pp. 246–254, 2006.

[9] C. C. Stichel, X. R. Zhu, V. Bader, B. Linnartz, S. Schmidt, and H. Lübbert, "Mono- and double-mutant mouse models of Parkinson's disease display severe mitochondrial damage," *Human Molecular Genetics*, vol. 16, no. 20, pp. 2377–2393, 2007.

[10] M. T. Lin and M. F. Beal, "Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases," *Nature*, vol. 443, no. 7113, pp. 787–795, 2006.

[11] B. B. Lowell and G. I. Shulman, "Mitochondrial dysfunction and type 2 diabetes," *Science*, vol. 307, no. 5708, pp. 384–387, 2005.

[12] S. Blanche, M. Tardieu, P. Rustin et al., "Persistent mitochondrial dysfunction and perinatal exposure to antiretroviral nucleoside analogues," *The Lancet*, vol. 354, no. 9184, pp. 1084–1089, 1999.

[13] D. C. Chan, "Mitochondria: dynamic organelles in disease, aging, and development," *Cell*, vol. 125, no. 7, pp. 1241–1252, 2006.

[14] E. A. Schon and G. Manfredi, "Neuronal degeneration and mitochondrial dysfunction," *The Journal of Clinical Investigation*, vol. 111, no. 3, pp. 303–312, 2003.

[15] G. C. Kujoth, A. Hiona, T. D. Pugh et al., "Medicine: mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging," *Science*, vol. 309, no. 5733, pp. 481–484, 2005.

[16] F. M. Yakes and B. Van Houten, "Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress," *Proceedings of the National Acadamy of Sciences of the United States of America*, vol. 94, no. 2, pp. 514–519, 1997.

[17] A. W. Linnane, S. Marzuki, T. Ozawa, and M. Tanaka, "Mitochondrial DNA mutations as an important contributor to ageing and degenerative diseases," *The Lancet*, vol. 1, no. 8639, pp. 642–645, 1989.

[18] T. B. Sherer, R. Betarbet, and J. T. Greenamyre, "Environment, mitochondria, and Parkinson's disease," *The Neuroscientist*, vol. 8, no. 3, pp. 192–197, 2002.

[19] D. A. Di Monte, "The environment and Parkinson's disease: is the nigrostriatal system preferentially targeted by neurotoxins?" *The Lancet Neurology*, vol. 2, no. 9, pp. 531–538, 2003.

[20] R. A. Rudel, D. E. Camann, J. D. Spengler, L. R. Korn, and J. G. Brody, "Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust," *Environmental Science & Technology*, vol. 37, no. 20, pp. 4543–4553, 2003.

[21] C. Sonnenschein and A. M. Soto, "An updated review of environmental estrogen and androgen mimics and antagonists," *The Journal of Steroid Biochemistry and Molecular Biology*, vol. 65, no. 1-6, pp. 143–150, 1998.

[22] A. Valavanidis, T. Vlahogianni, M. Dassenakis, and M. Scoullos, "Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants," *Ecotoxicology and Environmental Safety*, vol. 64, no. 2, pp. 178–189, 2006.

[23] S. Delacroix, C. Vogelsang, A. Tobiesen, and H. Liltved, "Disinfection by-products and ecotoxicity of ballast water after oxidative treatment - Results and experiences from seven years of full-scale testing of ballast water management systems," *Marine Pollution Bulletin*, vol. 73, no. 1, pp. 24–36, 2013.

[24] M. L. Hanson, P. K. Sibley, S. A. Mabury, K. R. Solomon, and D. C. Muir, "Trichloroacetic acid (TCA) and trifluoroacetic acid (TFA) mixture toxicity to the macrophytes Myriophyllum spicatum and Myriophyllum sibiricum in aquatic microcosms," *Science of the Total Environment*, vol. 285, no. 1-3, pp. 247–259, 2002.

[25] G. A. Kahrilas, J. Blotevogel, P. S. Stewart, and T. Borch, "Biocides in hydraulic fracturing fluids: A critical review of their usage, mobility, degradation, and toxicity," *Environmental Science & Technology*, vol. 49, no. 1, pp. 16–32, 2015.

[26] M. J. Plewa, Y. Kargalioglu, D. Vankerk, R. A. Minear, and E. D. Wagner, "Development of quantitative comparative cytotoxicity and genotoxicity assays for environmental hazardous chemicals," *Water Science and Technology*, vol. 42, no. 7-8, pp. 109–116, 2000.

[27] M. J. Plewa, E. D. Wagner, and S. D. Richardson, "TIC-Tox: A preliminary discussion on identifying the forcing agents of DBP-mediated toxicity of disinfected water," *Journal of Environmental Sciences*, vol. 58, pp. 208–216, 2017.

[28] F. Bove, Y. Shim, and P. Zeitz, "Drinking water contaminants and adverse pregnancy outcomes: a review," *Environmental Health Perspectives*, vol. 110, pp. 61–74, 2002.

[29] R. J. Bull, D. A. Reckhow, X. Li, A. R. Humpage, C. Joll, and S. E. Hrudey, "Potential carcinogenic hazards of non-regulated disinfection by-products: Haloquinones, halo-cyclopentene and

cyclohexene derivatives, N-halamines, halonitriles, and heterocyclic amines," *Toxicology*, vol. 286, no. 1-2, pp. 1–19, 2011.

[30] C. H. Jeong, E. D. Wagner, V. R. Siebert et al., "Occurrence and toxicity of disinfection byproducts in European drinking waters in relation with the HIWATE epidemiology study," *Environmental Science and Technology*, vol. 46, no. 21, pp. 12120–12128, 2012.

[31] M. Lynberg, J. R. Nuckols, P. Langlois et al., "Assessing exposure to disinfection by-products in women of reproductive age living in Corpus Christi, Texas and Cobb County, Georgia: Descriptive results and methods," *Environmental Health Perspectives*, vol. 109, no. 6, pp. 597–604, 2001.

[32] M. J. Nieuwenhuijsen, J. Grellier, R. Smith et al., "The epidemiology and possible mechanisms of disinfection by-products in drinking water," *Philosophical Transactions of the Royal Society A: Mathematical, Physical & Engineering Sciences*, vol. 367, no. 1904, pp. 4043–4076, 2009.

[33] Y. Woo, D. Lai, J. L. McLain, M. K. Manibusan, and V. Dellarco, "Use of mechanism-based structure-activity relationships analysis in carcinogenic potential ranking for drinking water disinfection by-products," *Environmental Health Perspectives*, vol. 110, no. 1, pp. 75–87, 2002.

[34] J. Choi and R. L. Valentine, "Formation of N-nitrosodimethylamine (NDMA) from reaction of monochloramine: A new disinfection by-product," *Water Research*, vol. 36, no. 4, pp. 817–824, 2002.

[35] A. D. Shah and W. A. Mitch, "Halonitroalkanes, halonitriles, haloamides, and N-nitrosamines: A critical review of nitrogenous disinfection byproduct formation pathways," *Environmental Science & Technology*, vol. 46, no. 1, pp. 119–131, 2012.

[36] V. K. Sharma, "Potassium ferrate(VI): an environmentally friendly oxidant," *Advances in Environmental Research*, vol. 6, no. 2, pp. 143–156, 2002.

[37] U. Von Gunten, "Ozonation of drinking water: part II. Disinfection and by-product formation in presence of bromide, iodide or chlorine," *Water Research*, vol. 37, no. 7, pp. 1469–1487, 2003.

[38] J. R. Hayes, L. W. Condie Jr., and J. F. Borzelleca, "Toxicology of haloacetonitriles," *Environmental Health Perspectives*, vol. 69, pp. 183–202, 1986.

[39] S. D. Richardson, "Disinfection by-products and other emerging contaminants in drinking water," *TrAC Trends in Analytical Chemistry*, vol. 22, no. 10, pp. 666–684, 2003.

[40] S. D. Richardson, M. J. Plewa, E. D. Wagner, R. Schoeny, and D. M. DeMarini, "Occurrence, genotoxicity, and carcinogenicity of regulated and emerging disinfection by-products in drinking water: a review and roadmap for research," *Mutation Research*, vol. 636, no. 1–3, pp. 178–242, 2007.

[41] R. Nilsson, M. Tasheva, and B. Jaeger, "Why different regulatory decisions when the scientific information base is similar?—Human risk assessment," *Regulatory Toxicology and Pharmacology*, vol. 17, no. 3, pp. 292–332, 1993.

[42] C. A. Damalas and I. G. Eleftherohorinos, "Pesticide exposure, safety issues, and risk assessment indicators," *International Journal of Environmental Research and Public Health*, vol. 8, no. 5, pp. 1402–1419, 2011.

[43] K. Kümmerer, "The presence of pharmaceuticals in the environment due to human use: present knowledge and future challenges," *Journal of Environmental Management*, vol. 90, no. 8, pp. 2354–2366, 2009.

[44] J. Bridges, "Human health and environmental risk assessment: The need for a more harmonised and integrated approach," *Chemosphere*, vol. 52, no. 9, pp. 1347–1351, 2003.

[45] S. J. Klaine, A. A. Koelmans, N. Horne et al., "Paradigms to assess the environmental impact of manufactured nanomaterials," *Environmental Toxicology and Chemistry*, vol. 31, no. 1, pp. 3–14, 2012.

[46] C. Kilkenny, W. Browne, I. C. Cuthill, M. Emerson, and D. G. Altman, "Animal research: Reporting in vivo experiments: The ARRIVE guidelines," *The Journal of Gene Medicine*, vol. 12, no. 7, pp. 561–563, 2010.

[47] G. T. Ankley, R. S. Bennett, R. J. Erickson et al., "Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment," *Environmental Toxicology and Chemistry*, vol. 29, no. 3, pp. 730–741, 2011.

[48] D. L. Villeneuve, D. Crump, N. Garcia-Reyero et al., "Adverse outcome pathway development II: Best practices," *Toxicological Sciences*, vol. 142, no. 2, pp. 321–330, 2014.

[49] D. L. Villeneuve, D. Crump, N. Garcia-Reyero et al., "Adverse outcome pathway (AOP) development I: Strategies and principles," *Toxicological Sciences*, vol. 142, no. 2, pp. 312–320, 2014.

[50] R. A. Becker, G. T. Ankley, S. W. Edwards et al., "Increasing scientific confidence in adverse outcome pathways: application of tailored bradford-hill considerations for evaluating weight of evidence," *Regulatory Toxicology and Pharmacology*, vol. 72, no. 3, pp. 514–537, 2015.

[51] H. F. Smyth, C. P. Carpenter, C. S. Weil, U. C. Pozzani, and J. A. Striegel, "Range-Finding Toxicity Data: List VI," *American Industrial Hygiene Association Journal*, vol. 23, no. 2, pp. 95–107, 1962.

[52] E. T. Kimura, D. M. Ebert, and P. W. Dodge, "Acute toxicity and limits of solvent residue for sixteen organic solvents," *Toxicology and Applied Pharmacology*, vol. 19, no. 4, pp. 699–704, 1971.

[53] M. J. Plewa, E. D. Wagner, M. G. Muellner, K. M. Hsu, and S. D. Richardson, "Comparative Mammalian Cell Toxicity of N-DBPs and C-DBPs," *Abstracts of Papers of the American Chemical Society*, vol. 233, p. 372, 2007.

[54] M. J. Plewa, M. G. Muellner, S. D. Richardson et al., "Occurrence, synthesis, and mammalian cell cytotoxicity and genotoxicity of haloacetamides: An emerging class of nitrogenous drinking water disinfection byproducts," *Environmental Science & Technology*, vol. 42, no. 3, pp. 955–961, 2008.

[55] S. A. Saghir and K. K. Rozman, "Kinetics of monochloroacetic acid at subtoxic and toxic doses in rats after single oral and dermal administrations," *Toxicological Sciences*, vol. 76, no. 1, pp. 51–64, 2003.

[56] H. K. Bhat, M. F. Kanz, G. A. Campbell, and G. A. Ansari, "Ninety day toxicity study of chloroacetic acids in rats," *Fundamental and Applied Toxicology*, vol. 17, no. 2, pp. 240–253, 1991.

[57] K. T. Kitchen and J. L. Brown, "Biochemical effects of 3 clorinated phenols in rat liver," *Toxicological & Environmental Chemistry*, vol. 16, no. 3, pp. 165–172, 1988.

[58] A. E. Munson, L. E. Sain, V. M. Sanders et al., "Toxicology of organic drinking water contaminants: trichloromethane, bromodichloromethane, dibromochloromethane and tribromomethane," *Environmental Health Perspectives*, vol. 46, pp. 117–126, 1982.

[59] I. Chu, V. Secours, I. Marino, and D. C. Villeneuve, "Acute toxicity of 4 trihalomethanes in male and female rats," *Toxicology and Applied Pharmacology*, vol. 52, no. 2, pp. 351–353, 1980.

[60] I. G. Kirkinezos and C. T. Moraes, "Reactive oxygen species and mitochondrial diseases," *Seminars in Cell & Developmental Biology*, vol. 12, no. 6, pp. 449–457, 2001.

[61] G. Lenaz, "The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology," *IUBMB Life*, vol. 52, no. 3–5, pp. 159–164, 2002.

[62] M. C. Carreras, M. C. Franco, J. G. Peralta, and J. J. Poderoso, "Nitric oxide, complex I, and the modulation of mitochondrial reactive species in biology and disease," *Molecular Aspects of Medicine*, vol. 25, no. 1-2, pp. 125–139, 2004.

[63] A. E. Ahmed, S. Jacob, G. A. Campbell, H. M. Harirah, J. R. Perez-Polo, and K. M. Johnson, "Fetal origin of adverse pregnancy outcome: The water disinfectant by-product chloroacetonitrile induces oxidative stress and apoptosis in mouse fetal brain," *Developmental Brain Research*, vol. 159, no. 1, pp. 1–11, 2005.

[64] V. K. Sharma, P. K. Dutta, and A. K. Ray, "Review of kinetics of chemical and photocatalytical oxidation of Arsenic(III) as influenced by pH," *Journal of Environmental Science and Health*, vol. 42, no. 7, pp. 997–1004, 2007.

[65] A. E. Ahmed, S. Jacob, and A. M. Nouraldeen, "Chloroacetonitrile (CAN) induces glutathione depletion and 8-hydroxylation of guanine bases in rat gastric mucosa," *Journal of Biochemical and Molecular Toxicology*, vol. 13, no. 3-4, pp. 119–126, 1999.

[66] J. Pals, M. S. Attene-Ramos, M. Xia, E. D. Wagner, and M. J. Plewa, "Human cell toxicogenomic analysis linking reactive oxygen species to the toxicity of monohaloacetic acid drinking water disinfection byproducts," *Environmental Science & Technology*, vol. 47, no. 21, pp. 12514–12523, 2013.

[67] J. Li, B. Moe, S. Vemula, W. Wang, and X.-F. Li, "Emerging disinfection byproducts, halobenzoquinones: effects of isomeric structure and halogen substitution on cytotoxicity, formation of reactive oxygen species, and genotoxicity," *Environmental Science & Technology*, vol. 50, no. 13, pp. 6744–6752, 2016.

[68] K. F. Ilett, W. D. Reid, I. G. Sipes, and G. Krishna, "Chloroform toxicity in mice: Correlation of renal and hepatic necrosis with covalent binding of metabolites to tissue macromolecules," *Experimental and Molecular Pathology*, vol. 19, no. 2, pp. 215–229, 1973.

[69] T. Ekström and J. Högberg, "Chloroform-induced glutathione depletion and toxicity in freshly isolated hepatocytes," *Biochemical Pharmacology*, vol. 29, no. 22, pp. 3059–3065, 1980.

[70] A. Reeve, E. Simcox, and D. Turnbull, "Ageing and Parkinson's disease: Why is advancing age the biggest risk factor?" *Ageing Research Reviews*, vol. 14, no. 1, pp. 19–30, 2014.

[71] D. E. Meyers, H. I. Basha, and M. K. Koenig, "Mitochondrial cardiomyopathy: Pathophysiology, diagnosis, and management," *Texas Heart Institute Journal*, vol. 40, no. 4, pp. 385–394, 2013.

[72] A. Nel, "Air pollution-related illness: effects of particles," *Science*, vol. 308, no. 5723, pp. 804–806, 2005.

[73] B. Halliwell, "Reactive oxygen species in living systems: Source, biochemistry, and role in human disease," *American Journal of Medicine*, vol. 91, no. 3 C, pp. S14–S22, 1991.

[74] D. Bagchi, M. Bagchi, E. A. Hassoun, and S. J. Stohs, "In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides," *Toxicology*, vol. 104, no. 1–3, pp. 129–140, 1995.

[75] D. Kulms, E. Zeise, B. Pöppelmann, and T. Schwarz, "DNA damage, death receptor activation and reactive oxygen species contribute to ultraviolet radiation-induced apoptosis in an essential and independent way," *Oncogene*, vol. 21, no. 38, pp. 5844–5851, 2002.

[76] T. D. Sharkey, "Effects of moderate heat stress on photosynthesis: Importance of thylakoid reactions, rubisco deactivation, reactive oxygen species, and thermotolerance provided by isoprene," *Plant, Cell & Environment*, vol. 28, no. 3, pp. 269–277, 2005.

[77] K. Apel and H. Hirt, "Reactive oxygen species: metabolism, oxidative stress, and signal transduction," *Annual Review of Plant Biology*, vol. 55, pp. 373–399, 2004.

[78] V. I. Bruskov, L. V. Malakhova, Z. K. Masalimov, and A. V. Chernikov, "Heat-induced formation of reactive oxygen species and 8-oxoguanine, a biomarker of damage to DNA," *Nucleic Acids Research*, vol. 30, no. 6, pp. 1354–1363, 2002.

[79] C. Carlson, S. M. Hussein, A. M. Schrand et al., "Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species," *The Journal of Physical Chemistry B*, vol. 112, no. 43, pp. 13608–13619, 2008.

[80] C. M. Sayes, A. M. Gobin, K. D. Ausman, J. Mendez, J. L. West, and V. L. Colvin, "Nano-C60 cytotoxicity is due to lipid peroxidation," *Biomaterials*, vol. 26, no. 36, pp. 7587–7595, 2005.

[81] H. Lujan and C. M. Sayes, "Cytotoxicological pathways induced after nanoparticle exposure: Studies of oxidative stress at the 'nano-bio' interface," *Toxicology Research*, vol. 6, no. 5, pp. 580–594, 2017.

[82] A. Bender, "TOM40 mediates mitochondrial dysfunction induced by alpha-synuclein accumulation in Parkinson's disease," *PLoS ONE*, vol. 8, no. 4, 2013.

[83] P. L. Olive and P. J. Johnston, "DNA damage from oxidants: Influence of lesion complexity and chromatin organization," *Oncology Research : Featuring Preclinical and Clinical Cancer Therapeutics*, vol. 9, no. 6-7, pp. 287–294, 1997.

[84] S. Toyokuni, "Reactive oxygen species-induced molecular damage and its application in pathology," *Pathology International*, vol. 49, no. 2, pp. 91–102, 1999.

[85] N. Launay, M. Ruiz, S. Fourcade et al., "Oxidative stress regulates the ubiquitin-proteasome system and immunoproteasome functioning in a mouse model of X-adrenoleukodystrophy," *Brain*, vol. 136, no. 3, pp. 891–904, 2013.

[86] N. S. Chandel, D. S. McClintock, C. E. Feliciano et al., "Reactive oxygen species generated at mitochondrial Complex III stabilize hypoxia-inducible factor-1α during hypoxia: a mechanism of O_2 sensing," *The Journal of Biological Chemistry*, vol. 275, no. 33, pp. 25130–25138, 2000.

[87] S.-J. Kim, P. Cheresh, R. P. Jablonski, D. B. Williams, and D. W. Kamp, "The role of mitochondrial DNA in mediating alveolar epithelial cell apoptosis and pulmonary fibrosis," *International Journal of Molecular Sciences*, vol. 16, no. 9, pp. 21486–21519, 2015.

[88] L. Li, S. Nadanaciva, Z. Berger et al., "Human A53T α-synuclein causes reversible deficits in mitochondrial function and dynamics in primary mouse cortical neurons," *PLoS ONE*, vol. 8, no. 12, Article ID e85815, 2013.

[89] R. J. Mailloux, S. L. McBride, and M.-E. Harper, "Unearthing the secrets of mitochondrial ROS and glutathione in bioenergetics," *Trends in Biochemical Sciences*, vol. 38, no. 12, pp. 592–602, 2013.

[90] N. D. Vaziri, X. Q. Wang, F. Oveisi, and B. Rad, "Induction of oxidative stress by glutathione depletion causes severe hypertension in normal rats," *Hypertension*, vol. 36, no. 4, p. 688, 2000.

[91] İ. S. Övey and M. Naziroğlu, "Homocysteine and cytosolic GSH depletion induce apoptosis and oxidative toxicity through cytosolic calcium overload in the hippocampus of aged mice: involvement of TRPM2 and TRPV1 channels," *Neuroscience*, vol. 284, pp. 225–233, 2015.

[92] C. Lu and J. S. Armstrong, "Role of calcium and cyclophilin D in the regulation of mitochondrial permeabilization induced by glutathione depletion," *Biochemical and Biophysical Research Communications*, vol. 363, no. 3, pp. 572–577, 2007.

[93] H. K. Baumgartner, J. V. Gerasimenko, C. Thorne et al., "Calcium elevation in mitochondria is the main Ca2+ requirement for mitochondrial permeability transition pore (mPTP) opening," *The Journal of Biological Chemistry*, vol. 284, no. 31, pp. 20796–20803, 2009.

[94] P. Bernardi, "Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore by the proton electrochemical gradient: evidence that the pore can be opened by membrane depolarization," *The Journal of Biological Chemistry*, vol. 267, no. 13, pp. 8834–8839, 1992.

[95] A. Almeida, S. J. R. Heales, J. P. Bolaños, and J. M. Medina, "Glutamate neurotoxicity is associated with nitric oxide-mediated mitochondrial dysfunction and glutathione depletion," *Brain Research*, vol. 790, no. 1-2, pp. 209–216, 1998.

[96] J. I. E. Bruce, "Plasma membrane calcium pump regulation by metabolic stress," *World Journal of Biological Chemistry*, vol. 1, no. 7, pp. 221–228, 2010.

[97] J. H. Santos, L. Hunakova, Y. Chen, C. Bortner, and B. Van Houten, "Cell sorting experiments link persistent mitochondrial DNA damage with loss of mitochondrial membrane potential and apoptotic cell death," *The Journal of Biological Chemistry*, vol. 278, no. 3, pp. 1728–1734, 2003.

[98] R. A. Ehlers, A. Hernandez, L. S. Bloemendal, R. T. Ethridge, B. Farrow, and B. M. Evers, "Mitochondrial DNA damage and altered membrane potential (Delta Psi) in pancreatic acinar cells induced by reactive oxygen species," *Surgery*, vol. 126, no. 2, pp. 148–155.

[99] Y. Du, Z. Ma, S. Lin et al., "Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease," *Proceedings of the National Acadamy of Sciences of the United States of America*, vol. 98, no. 25, pp. 14669–14674, 2001.

[100] D. Hernandez-Baltazar, M. E. Mendoza-Garrido, and D. Martinez-Fong, "Activation of GSK-3β and caspase-3 occurs in nigral dopamine neurons during the development of apoptosis activated by a striatal injection of 6-hydroxydopamine," *PLos One*, vol. 8, no. 8, Article ID e70951, 2013.

[101] S. Ares-Santos, N. Granado, I. Espadas, R. Martinez-Murillo, and R. Moratalla, "Methamphetamine causes degeneration of dopamine cell bodies and terminals of the nigrostriatal pathway evidenced by silver staining," *Neuropsychopharmacology*, vol. 39, no. 5, pp. 1066–1080, 2014.

[102] E. D. Crouser, "Mitochondrial dysfunction in septic shock and multiple organ dysfunction syndrome," *Mitochondrion*, vol. 4, no. 5-6, pp. 729–741, 2004.

Hindawi
Journal of Toxicology
Volume 2019, Article ID 8203832, 7 pages
https://doi.org/10.1155/2019/8203832

Research Article

Phytochemical Screening and Toxicological Study of *Aristolochia baetica* Linn Roots: Histopathological and Biochemical Evidence

Mohammed Bourhia,[1] Amal Ait Haj Said,[2] Ayoub Chaanoun,[3] Fatiha El Gueddari,[1] Abderrahim Naamane,[1] Laila Benbacer,[4] and Naima Khlil[1]

[1] *Laboratory of Chemistry-Biochemistry, Environment, Nutrition, and Health, Faculty of Medicine and Pharmacy of Casablanca, Hassan II University, 19 rue Tarik Ibn Ziad, Casablanca, Morocco*
[2] *Laboratory of Pharmacognosy, Faculty of Medicine and Pharmacy of Casablanca, Hassan II University, 19 rue Tarik Ibn Ziad, Casablanca, Morocco*
[3] *Laboratory of Health and Agri-Food, Faculty of Science and Technology, University Hassan I, Route de Casablanca Km. 3,5, BP 539, Settat, Morocco*
[4] *Life Science Division, National Centre for Energy, Sciences, and Nuclear Techniques, BP 1382, 10001 Rabat, Morocco*

Correspondence should be addressed to Mohammed Bourhia; bourhiamohammed@gmail.com

Received 10 July 2018; Revised 2 October 2018; Accepted 23 October 2018; Published 3 February 2019

Guest Editor: Wageh Sobhy Darwish

Aristolochia baetica (A. *baetica*) is a wild species of Aristolochiaceae family; its roots are used by Moroccan people against cancer for many years ago. The objective of the study was to investigate the phytochemical screening, acute and subacute toxicity of *A. baetica* roots growing in the north of Morocco. Qualitative and quantitative analyses of *A. baetica* roots were performed using standard methods; the acute toxicity of the root extract of the studied plant was assessed in mice by gavage of single doses of 1, 2, and 4 g/kg body weight for 14 days; by the time the subacute toxicity was done using repeated doses 1, 1.5, and 2 g/kg/day for 28 days. Histological changes and biochemical parameters as markers of kidney and liver function were evaluated. The results of phytochemical screening showed the presence of polyphenols, tannins, alkaloids, flavonoids, saponins, and the absence of anthraquinones, sterols, and terpenes. The results of acute toxicity showed the absence of mortality and signs of toxicity in groups treated with 1 and 2 g/kg; however, the clinical signs of toxicity were important and the rate of mortality was estimated at 16 % in the group treated with 4 g/kg. The results of subacute toxicity showed several changes of serum parameters registered in groups treated with 1.5 and 2 g/kg/day, respectively. The results showed also the absence of histological injuries in groups treated with 1 and 1.5 g/kg/day; meanwhile, the histological alterations were remarkable in treated group with the highest dose administered of 2 g/kg/day. The outcome of this work showed that the roots' extract of the studied plant was toxic in mice with repeated doses, but no toxic effect was observed with a single dose under 4g/kg.

1. Introduction

For many years ago, the medicinal plants have been largely used in the treatment of many diseases throughout the world; plants contain naturally a large variety of chemical substances with different pharmacological and biological activities. As reported in the literature, the percentage of Moroccan people using traditional medicines ranges from 50 to 75 % [1]; meanwhile, many other studies have shown that a huge quantity of herbs which used without scientific proof may overexert toxic effects [2].

A. baetica belongs to the Aristolochiaceae family, is a wild species used by the Moroccans against several diseases since ancient time; especially the roots prepared in water are used against cancer [3], and digestive diseases [4], the aerial parts are utilized to treat abortifacient, the flower parts are

used to treat rheumatic. The whole plant of *A. baetica* is also decocted in water and used as anti-inflammatory and antiseptic in many regions of Morocco [5]. As matter of fact the preparation including plants of genus Aristolochia is banned because of their toxicities due to aristolochic acids (AAs). The AAs case was detected at first in Belgium into a group of women patients who was affected by critical renal disease after ingesting the plant of *Aristolochia fangchi* for a long time [6]. Aristolochic acids have been recognized to be toxic for neurons [7], carcinogenic [8], and mutagenic [9]; the herbal remedies which contain the plant including genus Aristolochia have been banned in many countries throughout the world [10]. The responsible places of health agencies of Morocco declared in press that some cases of renal failures were registered due to adopting a preparation including Bereztem [11]; on the other hand some cases of death were recorded in Morocco due to using *A. longa* in the preparation against cancer [12].

It is kindly noted that the aim of this study was to evaluate the phytochemical screening, acute and subacute toxicity of *A. baetica* prepared in decoction; thus, different doses of the studied plant were administered to mice, biochemical parameters and histological changes were studied

2. Materials and Methods

2.1. Plant Material. Roots of *A. baetica* were harvested from Meknes region a city in Morocco about 150 km east of Moroccan capital (Rabat) in October 2016. A voucher specimen were taxonomically identified and deposited in the Herbarium of Scientific Institute of University Mohammed V–Rabat–Morocco. *A. baetica roots* were washed with water, dried at room temperature, and ground into a fine powder using an electric mixer.

2.2. Aqueous Extract Preparation. The aqueous extract of *A. baetica* was prepared following the standard traditional method described in the literature [13]. The powder of roots was first boiled for 20 min at 100°C. Thereafter, the mixture obtained was cooled at room temperature and then centrifuged, and the supernatant was filtered using Whatman filter paper. The filtrate was concentrated in a rotary vacuum evaporator. The crude extract reconstituted on a daily basis in distilled water for final concentrations required for oral administration [14].

2.3. Preliminary Qualitative Phytochemical Screening. The plant material was subjected to qualitative phytochemical screening in order to qualitatively determine some type of interested phytoorganic constituents which are responsible for biological activities, alkaloids, flavonoids, polyphenols, anthraquinones, saponins, tannins, sterols, and terpenes which were the major cheeked groups using standard methods.

2.4. Animal Material. Adult Swiss albino mice weighing approximately 25 g were used for both acute and subacute toxicity; the mice were purchased from the animal colony of Pasteur Institute (Casablanca, Morocco). All animals were kept in polypropylene cages. The animals were acclimatized for one week under laboratory conditions of regular light/dark cycles (12/12 h) and temperature (24 ± 2°C). The animals had free access to tap water and normal pellet diet [15].

2.5. Study of Acute Toxicity. A total of 24 male adult mice were randomly divided into 4 experimental groups of 6 mice each; the animals were grouped according to selected doses of plant extract, one control group and three treatments. After fasting overnight, the aqueous extract of the plant roots was administered to each treatment group at single doses of 1, 2, and 4 mg/kg body weight; by the time the control group received an equivalent volume of distilled water (vehicle). After treatment, the mice were observed individually for clinical symptoms, mortality, and changes in general behavior during the period of treatment [16]. This study was conducted according to the Organization for Economic Cooperation and Development (OECD) Guideline No. 425 [17].

2.6. Study of Subacute Toxicity. The subacute toxicity study was effectuated according to the Organization for Economic Cooperation and Development (OECD) Guideline No. 407 [18]. A total of 24 male adult mice were randomly segregated into 4 groups of 6 mice per group for each; the mice were grouped according to selected doses of plant extract chosen to be tested, one control group and three treatments. Animals in treatment groups received repeatedly *B. dioica* root extract at doses of 1, 1.5, and 2 mg/kg/day for 28 days; by the time the control group received an equivalent volume of distilled water (vehicle). During the treatment period, the weight of animals was measured once a week. Animals were also observed for signs of toxicity, mortalities, changes in general behavior, and changes in physical appearance [19].

2.7. Biochemical Examination. On the 28 the day, all surviving animals were deprived of food overnight and sacrificed for blood collection. Heparin Tubes containing collected blood sample were centrifuged at 6000 rpm at 4°C for 20 min in order to obtain the serum. The measurement was effectuated according to the method described by Taj et al. (2014) [20], using an automated analyzer (roche cobas mira plus, Switzerland). The clinical biochemistry parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), urea, and creatinine.

2.8. Histopathological Evaluation. At the end of the treatment period, the major organs (liver and kidney) were removed for histopathological examination. The organs were carefully fixed in 10 % buffered formalin (pH 7.4). After fixation, tissue specimens were dehydrated in a graded series of ethanol (70–100%). After organization cropping and paraffin embedding, tissue specimens were stained with Haematoxylin and Eosin (H&E) prior to microscopic examination. The pathological examination of liver and kidneys tissue was carried out by a pathologist using a light microscope. The

Table 1: Phytochemical screening of *A. baetica* roots.

Polyphenols	+++
Alkaloids	+++
Flavonoids	+++
Anthraquinone	-
sterols and terpenes	-
saponins	++
Tannins	+++

+++: strong positive test; ++ positive test; +: low positive test; –: negative test.

microscopic features of examined organs of treated groups were compared to the control group [21].

2.9. Statistical Analysis. All quantitative data were expressed as the means ± SD (standard deviation). Statistical significance between the means of control and treated groups was determined by one-way ANOVA using GraphPad Prism 7 software. The means were pairwise compared using Tukey test and the differences were considered statistically significant at p less than 0.05.

3. Results

3.1. Qualitative Phytochemical Screening. The findings of preliminary qualitative phytochemical screening of *A. baetica* roots were shown in Table 1; they revealed the presence of flavonoids, polyphenols, alkaloids, tannins, and saponins and the absence of anthraquinone, sterols, and terpenes.

3.2. Acute Toxicity. During the acute toxicity study, no mortalities or signs of toxicity occurred in mice treated with a dose less than 4 g/kg; the mice were only characterized by an accelerated running about 3 to 5 min. However, the treatment with a dose of 4g/kg was responsible for shortness of breath, abnormal locomotion, hypoactivity, tending to deepen and be gentle, salivation, lack of appetite, lethargy, reversal reflection, occasional convulsion, and 16 % of deaths.

3.3. Subacute Toxicity. The general behavior of the mice and signs of toxicity were observed considering the mortality during the 28 days of feeding the aqueous extract; during the whole period of dosing, no visible toxic effects were noted in groups treated with 1 and 1.5 g/kg/day. From the 2nd week of treatment the mice treated with 2g/kg/day represented abnormal locomotion, ataxia, anorexia, hypoactivity and depression.

3.3.1. Effect of Aqueous Extract on the Mice Weight during the Treatment Period. During the treatment period, we did not note a significant change in the weight of treated mice with 1 and 1.5 g/kg/day body weight (group A and B) compared to the control group ($p>0.05$). This slight variation in weight may be due to the nervousness and stress of animals during and after swabbing. On the other hand, the treated mice with dose of 2 g/kg/day (group C) caused a weight loss from the second week of treatment, which became significant after 20 and 28 days of treatment ($p*<0.05$) (Figure 1).

3.3.2. Effect of Aqueous Extract on Biochemical Parameters. Some of the biochemical markers of liver function such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and kidney function such as urea, creatinine and lactate dehydrogenase (LDH) as a ubiquitous enzyme were measured. The results are represented in Figure 2.

Regarding the liver markers, the biochemical results showed a significant increase of AST dosed in group C (2 g/kg/day) compared to that recorded in the control group ($p**<0.05$). However, there is no significant change observed in groups A and B treated, respectively, with 1 and 1.5 g/kg/day) ($p>0.05$). regarding ALT transaminases, there is insignificant increase registered in groups A and B treated, respectively, with 1 and 1.5 g/kg/day compared to the control group; meanwhile we note a significant increase in group C (2 g/kg/day) ($p** <0.05$).

Regarding the renal markers, the creatinine concentration was increased in group B (1.5 g/kg/day) compared to the control group ($p*<0.05$). Meanwhile, there is no significant increase noted in group A (1 g/kg/day) and C (2g/kg/day), considering urea, the results showed insignificant change in all treated groups compared to the control lot ($p>0.05$). In regard to ubiquitous enzyme (LDH), we did not register any significant increase in groups A and B treated with 1 g/kg/day and 1.5 g/kg/day, respectively, compared to the control group ($p>0.05$). However, a significant increase was observed in group C treated with 2 g/kg/day ($p**<0.05$)

3.3.3. Histopathological Changes

3.3.4. Kidney. The histopathological examinations of the renal tissue showed that no histopathological changes occurred in the kidney of group A (1 g/kg/day); however, the renal tissue of groups treated with doses 1.5 and 2g/kg/day represented renal necrosis, inflammatory infiltrate, cortical necrosis, and tubular degeneration; the major results are summarized in Figure 3.

(A) Renal necrosis, (B) cortical necrosis, and (C) minimal inflammatory infiltrate.

3.3.5. Liver. The microscopic observation showed that no remarkable histopathological changes occurred in the liver of treated groups with doses of 1 and 1.5 g/kg/day; on the other hand, the renal tissue of group C (2g/kg/day) was described by inflammatory infiltrate, hepatic necrosis, and hepatic cholestasis; the major results are summarized in Figure 4.

(A) Inflammatory infiltrate and hepatic necrosis, (B) hepatic necrosis, and (C) minimal inflammatory infiltrate.

4. Discussion

Herbal medicines are largely appreciated by the public because it is believed to have no side effects due to their natural origins and are often seen as safe food supplements and not drugs. Medicinal plants are often self-prescribed by herbalist without control and review in terms of posology, manner, and frequency of administration. In reality, the

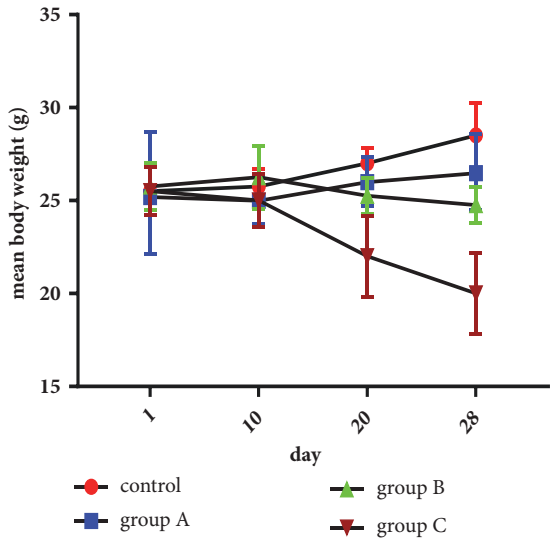


Figure 1: Changes of mice weight during the treatment period with doses 1 g/kg/day (group A), 1.5 g/kg/day (group B), and 2 g/kg/day (group C); results represent the means ± SD (standard deviation).

chemicals in the medicinal plant may be naturalistic to the plant itself, but they are not naturalistic to the human body. Truly any chemical compound with therapeutic effect has a possibility of being erroneously prescribed or overdosed.

The phytochemical profile of the plant extract revealed that the presence of alkaloids, polyphenols, flavonoids, tannins, and saponins could be the responsible compounds for the toxic effect of the studied plant. According to results of acute toxicity the rate of mortality was estimated at 16 % due to highest dose feed (4g/kg); considering the scale of Viala (1998) [22], the aqueous extract of roots decoction could be not or very little toxic [23]. On the other hand, there is no correlation between LD_{50} and aristolochic acids content recorded in the acute toxicity of organic extract of *Aristolochia manshuriensis* as reported in the literature [23]. Hence, these results suggest that other components in the extract may be dominating the acute toxicity.

Under the conditions of acute and subacute toxicity of roots decoction of *A. baetica,* the clinical signs observed such as hypoactivity and weight loss may be related to lack of appetite [6], lethargy, salivation, and anorexia which could be attributed to properties of the studied plant [24]; the seizures listed showed us the probable neurotoxic effect of roots decoction of *A. baetica* [25]. Our findings were compared to those reported in the study of subacute toxicity of *A. fructus* [19], in which it was reported that the animals feed with 22.2 g/kg of plant extract of *A. fructus* were affected by phenomenon, convulsion, reversal reflection and shortness of breath, dim and rough hair, and the ptosis closing.

Considering the findings of biochemical parameters, increased plasmatic level of urea and creatinine indicated that the roots' decoction of *A. Baetica* proved to be toxic [26]. On plasma level, we also noticed an increase in LDH (a soluble enzyme found normally inside every living cell) level into the surrounding extracellular space compared to the control mice, and this put us to think about the cell damage effects of roots decoction of *A. baetica* [27]. These obtained findings were supported by others reported in the toxicity studies conducted by Cherif and his collaborators, in which it was reported that the administration of aqueous extract of *Aristolochia longa* roots for 28 days affects the biochemical parameters in mice with a dose of 2.5 g/kg/day body weight [28]. The biochemical findings (disturbance of biochemical parameters) are confirmed by those obtained from histological examination of kidney and liver. Moreover, our results were compared to those discussed in the chronic toxicity of *A. manshuriensis* in which the authors reported that the absence of any significant biochemical variation after feeding mice with organic extract for eight weeks [14–23]. Thus, the organic extract of *A. manshuriensis* was responsible for hepatic necrosis [19]. Many studies showed that the oral administration of 2.5g/kg/day of the aqueous extract of *A. longa* induces histological injuries in the liver and kidneys, such as altered tissue architecture, lymphocytic infiltrates, foci of hemorrhage, hepatic-cholestasis, tubular necrosis, and cell congestion. Our results of histopathological changes such as renal necrosis, cortical necrosis, inflammatory infiltrate, and hepatic necrosis were supported by those obtained from biochemical evaluation; these findings were similar to those reported in the study of Cherif et al. [27–29]. Furthermore, our results are also comparable to those reported in the literature in which it was stated that the subacute toxicity of aqueous extracts of *Aristolochia Fructus* revealed a dose-dependent relationship of nephrotoxicity. However, the safe clinical dose of *A. Fructus* was 3.0 g/day for adults [19].

Taking into account the abundance of inflammatory infiltrate in two examined organs and especially into surrounding lesions, the histopathological results recorded during this work could be attributed to immunomodulatory properties of roots extract of *A. baetica* which might trigger an autoimmune response in the toxic lesions [29]. In point of fact, several studies have shown that ingestion of medicinal plants

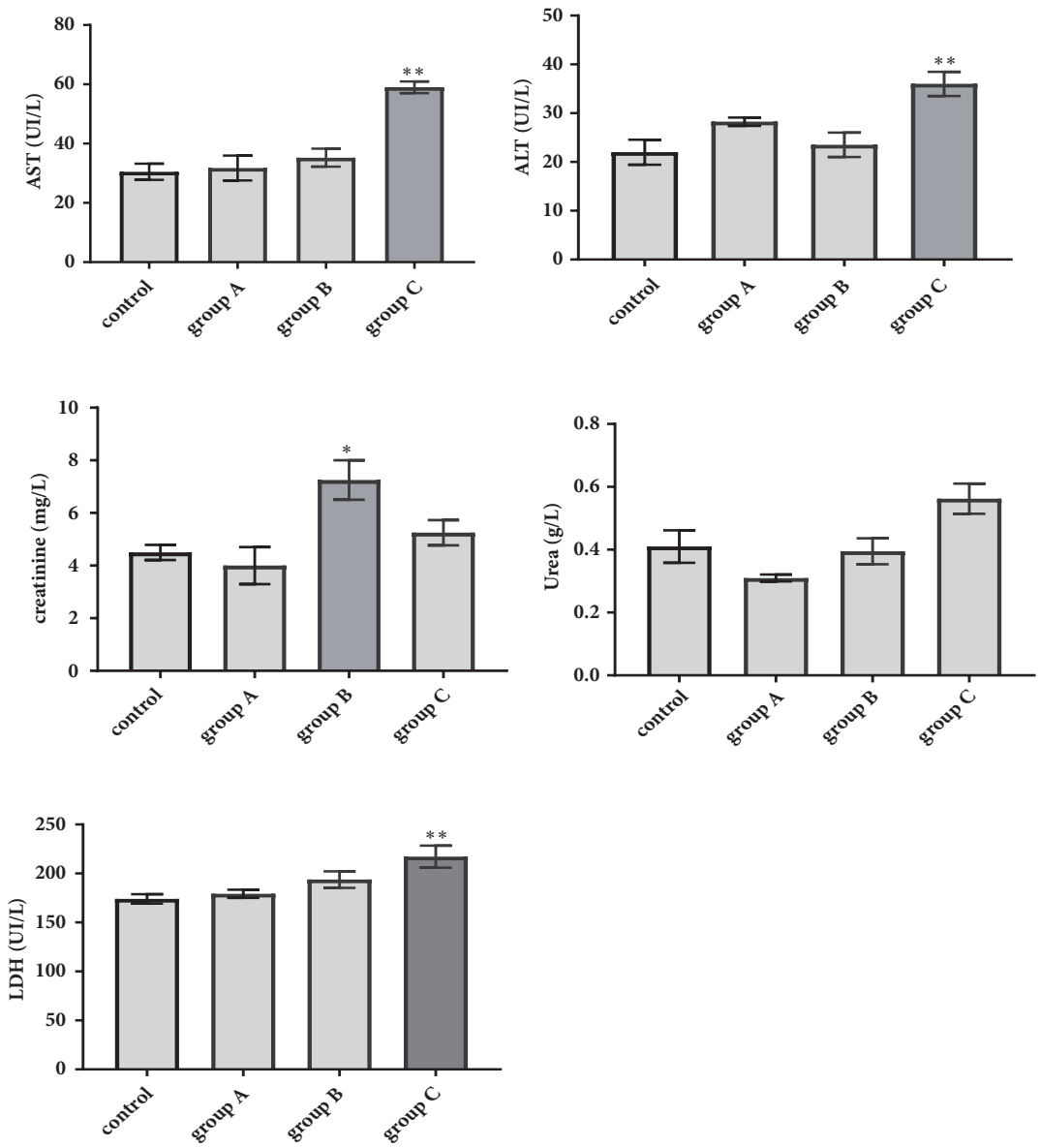


Figure 2: Effect of the plant extract at doses 1 g/kg/day (group A), 1.5 g/kg/day (group B), and 2 g/kg/day (group C) on the transaminases (ALT; AST), urea, creatinine, and lactate dehydrogenase (LDH) after the end of the experiment; results represent the means ± SD (standard deviation).

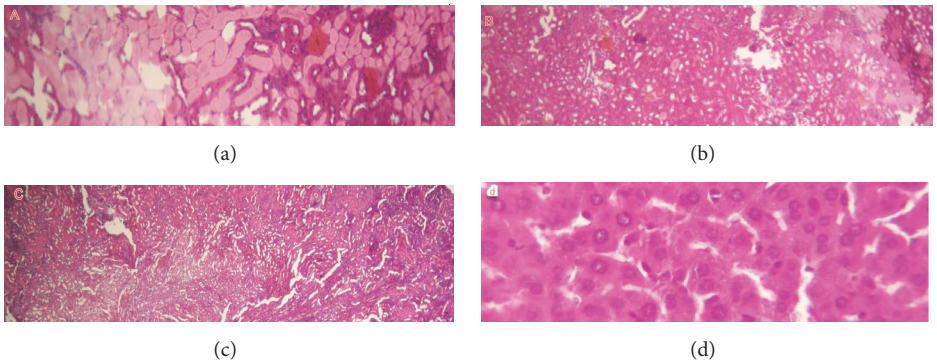


Figure 3: Histologic section of kidney tissue of control and treated mice (section of parenchyma stained with H&E, x 40). (a), (b), and (c) are histologic sections of kidney tissue of treated mice with 1.5 and 2 g/kg/day; (d) is a section of kidney tissue of control mice.

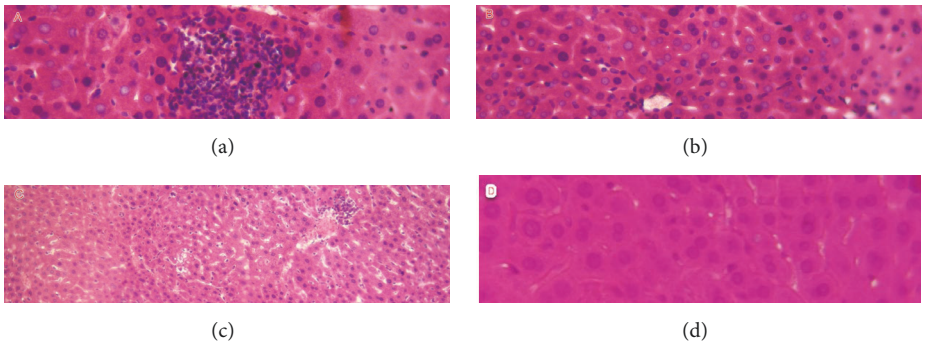


Figure 4: Histologic section of liver tissue of control and treated mice (section of parenchyma stained with H&E, x 40). (a), (b), and (c) are histologic sections of liver tissue of treated mice with 1.5 and 2 g/kg/day; (d) is a section of liver tissue of control mice.

including genus Aristolochia could be responsible for severe renal injury, including renal interstitial fibrosis [30, 31]. It should be noted that *A. baetica* and 31 related species are recognized to contain Aristolochic acids [5].

5. Conclusion

According to results of acute toxicity reported in this work, the aqueous extract of *A. baetica* was little toxic; meanwhile, the same extract showed high toxicity on serum parameters and histologic tissues when ingested for a long time under the subacute toxicity conditions. Considering our outcome of this study, we need to pay more attentions to the biodiversity of plants for safety control

Data Availability

(1) Previously reported "Immunostimulatory Potential of *Aristolochia longa* L. Induced Toxicity on Liver, Intestine and Kidney in Mice" data were used to support this study and are available at A Histopathological analyses of in vivo antitumor effect of an aqueous extract of Aristolochia longa used in cancer treatment in traditional medicine in Morocco', Int J Plant Res, vol. 2, pp. 31–35, 2012. These prior studies (and datasets) are cited at relevant places within the text as reference [29]. (2) Previously reported "Late Onset of Bladder Urothelial Carcinoma after Kidney Transplantation for End-Stage Aristolochic Acid Nephropathy" data were used to support this study and are available at 10.1053/j.ajkd.2007.11.015. These prior studies (and datasets) are cited at relevant places within the text as reference [7]. (3) Previously reported "Aristolochic Acid Induces Proximal Tubule Apoptosis and Epithelial to Mesenchymal Transformation" data were used to support this study and are available at 10.1038/sj.ki.5002714. These prior studies (and datasets) are cited at relevant places within the text as reference [12]. (4) Previously reported "Acute Toxicity Evaluation of Ethanolic Extract of *Aristolochia albida* Duch. Leaves on Wistar Rats Liver and Kidney Functions" data were used to support this study and are available at 10.22159/ijpps.2017v9i7.16887. These prior studies (and datasets) are cited at relevant places within the text as reference [19]. (5) Previously reported "Studies on the Toxicity of Aristolochia manshuriensis (Guanmuton)" data were used to support this study are available at DOI: 10.1016/j.tox.2004.01.026. These prior studies (and datasets) are cited at relevant places within the text as reference [23]. (6) Previously reported "Toxic Effects of Some Medicinal Plants Used in Moroccan Traditional Medicine" data were used to support this study and are available at Moroc. J Biol, vol. 2, no. 3, pp. 21–30, 2006. These prior studies (and datasets) are cited at relevant places within the text as reference [1].

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors thank the staff of Specialized Pathology Center (Casapath) for their contributions.

References

[1] M. Bnouham, F. Z. Merhfour, and M. Elachoui, "Toxic effects of some medicinal plants used in Moroccan traditional medicine," *Moroc. J Biol*, vol. 2, no. 3, pp. 21–30, 2006.

[2] M. Larhsini, "Biological and Toxicological Properties of Moroccan Plant Extracts: Advances in Research," *Modern Phytomedicine: Turning Medicinal Plants into Drugs*, pp. 123–136, 2006.

[3] A. Ouarghidi, G. J. Martin, B. Powell, G. Esser, and A. Abbad, "Botanical identification of medicinal roots collected and traded in Morocco and comparison to the existing literature," *Journal of Ethnobiology and Ethnomedicine*, vol. 9, no. 1, article no. 59, 2013.

[4] M. RHATTAS, A. DOUIRA, and L. ZIDANE, "Étude ethnobotanique des plantes médicinales dans le Parc National de Talassemtane (Rif occidental du Maroc)," *Journal of Applied Biosciences*, vol. 97, no. 0, p. 9187, 2016.

[5] W. Chaouki, D. Y. Leger, J. Eljastimi, J.-L. Beneytout, and M. Hmamouchi, "Antiproliferative effect of extracts from Aristolochia baetica and Origanum compactum on human breast cancer cell line MCF-7," *Pharmaceutical Biology*, vol. 48, no. 3, pp. 269–274, 2010.

[6] J. L. Vanherweghem, M. Depierreux, C. Tielemans et al., "Rapidly progressive interstitial renal fibrosis in young women:

association with slimming regimen including Chinese herbs," *The Lancet*, vol. 341, no. 8842, pp. 387–391, 1993.

[7] A. Lemy, K. M. Wissing, S. Rorive et al., "Late onset of bladder urothelial carcinoma after kidney transplantation for end-stage aristolochic acid nephropathy: a case series with 15-year follow-up," *American Journal of Kidney Diseases*, vol. 51, no. 3, pp. 471–477, 2008.

[8] C.-L. Cheng, K.-J. Chen, P.-H. Shih et al., "Chronic renal failure rats are highly sensitive to aristolochic acids, which are nephrotoxic and carcinogenic agents," *Cancer Letters*, vol. 232, no. 2, pp. 236–242, 2006.

[9] N. Feldmeyer, H. H. Schmeiser, K.-R. Muehlbauer et al., "Further studies with a cell immortalization assay to investigate the mutation signature of aristolochic acid in human p53 sequences," *Mutation Research - Genetic Toxicology and Environmental Mutagenesis*, vol. 608, no. 2, pp. 163–168, 2006.

[10] C. Zhang, X. Wang, M. Shang et al., "Simultaneous determination of five aristolochic acids and two aristololactams in *Aristolochia* plants by high-performance liquid chromatography," *Biomedical Chromatography*, vol. 20, no. 4, pp. 309–318, 2006.

[11] I. Tazi, H. Nafil, and L. Mahmal, "Fatal renal failure due to self administration of Aristolochia Longa after treatment with chemotherapy.," *Arab Journal of Nephrology and Transplantation*, vol. 5, no. 1, pp. 54-55, 2012.

[12] A. A. Pozdzik, I. J. Salmon, F. D. Debelle et al., "Aristolochic acid induces proximal tubule apoptosis and epithelial to mesenchymal transformation," *Kidney International*, vol. 73, no. 5, pp. 595–607, 2008.

[13] E. Ngo Bum, G. S. Taiwe, F. C. O. Moto et al., "Anticonvulsant, anxiolytic, and sedative properties of the roots of Nauclea latifolia Smith in mice," *Epilepsy & Behavior*, vol. 15, no. 4, pp. 434–440, 2009.

[14] F. F. Guinnin, J. R. Klotoe, and J. M. Ategbo, "Acute toxicity evaluation of ethanolic extract of aristolochia albida duch. leaves on wistar rats liver and kidney functions," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 9, no. 7, p. 35, 2017.

[15] Y. Wasihun, T. Adraro, and S. Ali, "Evaluation of Antibacterial Activity and Phytochemical Constituents of Leaf Extract of Lippia Adoensis," *Asia Pacific Journal of Energy and Environment*, vol. 1, no. 1, p. 45, 2014.

[16] H. A. A. Twaij, A. Kery, and N. K. Al-Khazraji, "Some pharmacological, toxicological and phytochemical investigations on *Centaurea phyllocephala*," *Journal of Ethnopharmacology*, vol. 9, no. 2-3, pp. 299–314, 1983.

[17] *in. OECD Guidelines for the Testing of Chemicals. Test Guideline 407. Repeated Dose 28-day Oral Toxicity Study in Rodents*, OECD (Organization for Economic Cooperation Development, 1998.

[18] *in. OECD Guidelines for the Testing of Chemicals. Test Guideline 425. Acute Oral Toxicity: Up-and- Down Procedure*, OECD (Organization for Economic Cooperation Development, Up-and- Down Procedure, 2008.

[19] J.-B. Yuan, Q. Huang, G. Ren et al., "Acute and subacute toxicity of the extract of Aristolochiae Fructus and honey-fried Aristolochiae Fructus in rodents," *Biological & Pharmaceutical Bulletin*, vol. 37, no. 3, pp. 387–393, 2014.

[20] D. Taj, H. Khan, V. Sultana, J. Ara, and S. Ehteshamul-Haque, "Antihepatotoxic effect of golden berry (Physalis peruviana Linn.) in carbon tetrachloride (CCl4) intoxicated rats," *Pakistan Journal of Pharmaceutical Sciences*, vol. 27, no. 3, pp. 491–494, 2014.

[21] A. Figueiredo-Fernandes, J. V. Ferreira-Cardoso, S. Garcia-Santos et al., "Histopathological changes in liver and gill epithelium of Nile tilapia, Oreochromis niloticus, exposed to waterborne copper," *Pesquisa Veterinária Brasileira*, vol. 27, no. 3, pp. 103–109, 2007.

[22] A. Viala, *Elements de toxicologie*, Lavoisier u.a, Paris, 1998.

[23] S.-L. Hu, H.-Q. Zhang, K. Chan, and Q.-X. Mei, "Studies on the toxicity of Aristolochia manshuriensis (Guanmuton)," *Toxicology*, vol. 198, no. 1-3, pp. 195–201, 2004.

[24] M. Depierreux, B. Van Damme, K. Vanden Houte, and J. L. Vanherweghem, "Pathologic Aspects of a Newly Described Nephropathy Related to the Prolonged Use of Chinese Herbs," *American Journal of Kidney Diseases*, vol. 24, no. 2, pp. 172–180, 1994.

[25] S. R. Norrby, "Neurotoxicity of carbapenem antibiotics: Consequences for their use in bacterial meningitis," *Journal of Antimicrobial Chemotherapy*, vol. 45, no. 1, pp. 5–7, 2000.

[26] S. Shibutani, H. Dong, N. Suzuki, S. Ueda, F. Miller, and A. P. Grollman, "Selective toxicity of aristolochic acids I and II," *Drug Metabolism and Disposition*, vol. 35, no. 7, pp. 1217–1222, 2007.

[27] H.-R. Chang, J.-D. Lian, C.-W. Lo, H.-P. Huang, and C.-J. Wang, "Aristolochic acid-induced cell cycle G1 arrest in human urothelium SV-HUC-1 cells," *Food and Chemical Toxicology*, vol. 45, no. 3, pp. 396–402, 2007.

[28] H. S. Cherif, F. Saidi, H. Boutoumi, A. Rouibi, and C. Chaouia, "Identification et caracterisation de quelques composes chimiques chez Aristolochia longa L," *Agric. Agric. Pract. Sci. J*, vol. 71, 2010.

[29] G. Benzakour, M. Amrani, and M. Oudghiri, "A Histopathological Analyses of in vivo Anti-tumor Effect of an Aqueous Extract of Aristolochia longa Used in Cancer Treatment in Traditional Medicine in Morocco," *International Journal of Plant Research*, vol. 2, no. 2, pp. 31–35, 2012.

[30] G. M. Lord, T. Cook, V. M. Arlt, H. H. Schmeiser, G. Williams, and C. D. Pusey, "Urothelial malignant disease and Chinese herbal nephropathy," *The Lancet*, vol. 358, no. 9292, pp. 1515-1516, 2001.

[31] V. Kumar, Poonam, A. K. Prasad, and V. S. Parmar, "Naturally occurring aristolactams, aristolochic acids and dioxoaporphines and their biological activities," *Natural Product Reports*, vol. 20, no. 6, pp. 565–583, 2003.

Hindawi
Journal of Toxicology
Volume 2019, Article ID 2345283, 14 pages
https://doi.org/10.1155/2019/2345283

Review Article

Environmental Chemical Contaminants in Food: Review of a Global Problem

Lesa A. Thompson [1] **and Wageh S. Darwish** [2,3]

[1] *Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060-0819, Japan*
[2] *Laboratory of Advanced Lipid Analysis, Department of Health Sciences and Technology, Faculty of Health Sciences, Hokkaido University, Sapporo 060-0812, Japan*
[3] *Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt*

Correspondence should be addressed to Lesa A. Thompson; lesa.thompson@vetmed.hokudai.ac.jp

Received 19 September 2018; Accepted 5 December 2018; Published 1 January 2019

Academic Editor: Valerio Matozzo

Contamination by chemicals from the environment is a major global food safety issue, posing a serious threat to human health. These chemicals belong to many groups, including metals/metalloids, polycyclic aromatic hydrocarbons (PAHs), persistent organic pollutants (POPs), perfluorinated compounds (PFCs), pharmaceutical and personal care products (PPCPs), radioactive elements, electronic waste, plastics, and nanoparticles. Some of these occur naturally in the environment, whilst others are produced from anthropogenic sources. They may contaminate our food—crops, livestock, and seafood—and drinking water and exert adverse effects on our health. It is important to perform assessments of the associated potential risks. Monitoring contamination levels, enactment of control measures including remediation, and consideration of sociopolitical implications are vital to provide safer food globally.

1. Introduction

Chemical contamination is a global food safety issue. There are many potentially toxic substances in the environment which may contaminate foods consumed by people. They include inorganic and organic substances and may originate from a wide range of sources (Figure 1 shows the pathway of contaminants through the environment). This review is restricted to chemical contamination of foods and does not address biological or physical hazards.

In certain instances, the source of contaminants may be the environment. This is the case for metals such as lead and mercury, dioxins, and polychlorinated biphenyls (PCBs). Agricultural use of pesticides may lead to food contamination. Similarly, drugs used in both people and animals may contaminate waterways and pose a health risk to consumers. Additionally, food packaging methods may be a source of contamination, so-called "migrants" leaching from packing materials. These contaminants may cause acute or chronic toxic effects. Toxicity may relate to the route of exposure and dose, and personal characteristics such as age and health condition may affect the individual's susceptibility.

Due to the nature of contamination, some food products may be more contaminated than others. This may be due to several factors such as varying exposure to pesticides, differences in plant uptake mechanisms from the environment, or contaminants from food packaging [1, 2]. Dietary make-up will affect an individual's exposure to these contaminants. For example, nursing neonates have a high intake of contaminants that are excreted in breast milk [3]. Exposure at different life stages may result in different toxic effects as well. For example, prenatal exposure to persistent organic pollutants has been linked to an increase in childhood obesity and increased blood pressure [4].

For many food items—including vegetables, fish, and other seafood—human health risk assessment data is available after analysis of available foods [5–7]. Urban farms and gardens may pose additional risks due to contaminants such as metals [8, 9]. Furthermore, drinking water may become contaminated [10, 11]. Xenoestrogenic compounds have even

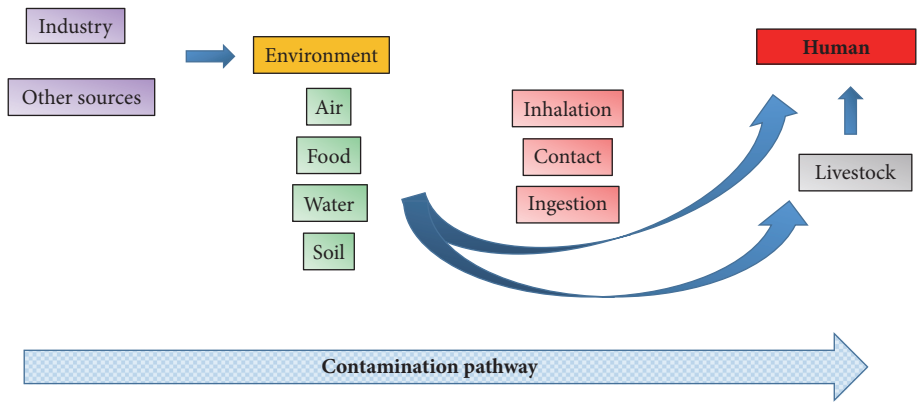


Figure 1: Sources of environmental contaminants in human foods.

been detected in rainwater [12]. Water contamination may also result in pollution of marine biota, affecting suitability for consumption of seafood [13]. Consequently individuals with high consumption of seafood will intake higher levels of such contaminants. Occupational exposure will not be discussed in detail in this review, but workers may have increased risk of exposure to certain contaminants, for example, car repair workers with lead on their hands which they ingest after hand-to-mouth contact [14].

For most contaminants, there is no completely safe dose level. However, for many, acceptable levels have been calculated—levels below which signs of toxicity should not be evident. Toxic effects seen depend on the contaminant in question, the dose received, and the individual. For example, many contaminants have been linked to an increased risk of cancer. Skin cancer has been associated with long-term exposure to drinking water contaminated by arsenic, gastric cancer with lead contamination, and liver cancer with consumption of grain contaminated by mercury [15–17]. Our understanding of the health risks from combined exposures to more than one contaminant and the means by which we can assess such interactions is lacking [18].

Monitoring programmes are in place both nationally and globally to monitor such contamination in order to assess food safety. However, it is important to note that such monitoring cannot completely preclude supply of contaminated food to consumers. The role of such programmes is to check that food and water contamination levels are below those deemed "unsafe." To this end, many governmental and non-governmental organizations strive through risk assessments to ascertain what levels of contamination are acceptable for products destined for human consumption. In addition, national and international policies are in place to reduce contamination. For example, under the Stockholm Convention on Persistent Organic Pollutants, production and use of such substances are eliminated or restricted. This international treaty came into force in 2004 and currently has 152 signatories from 182 parties [19]. The Codex Alimentarius describes international food standards, setting permitted maximum levels (ML) for contaminants in foods based on risk assessment and scientific evidence [20]. The Codex Committee on Contaminants in Food (CCCF) is a global forum, but it can be difficult to compromise national legislations and harmonize global standards. Lists of contaminants also undergo risk assessment by the Joint FAO/WHO Expert Committee on Food Additives [21, 22]. Recommendations are made for standards such as provisional maximum tolerable daily intake (PMTDI) or provisional tolerable weekly intake (PTWI). These are usually calculated based on chronic toxicity data, and thus it may also be useful to consider acute reference doses (ARfDs). The Codex Committee on Pesticide Residues (CCPR) has established maximum residue limits (MRLs) for over 5,000 pesticide residues [23]. This committee also considers reports from the FAO/WHO Meeting on Pesticide Residues (JMPR), which estimates MRLs and acceptable daily intakes (ADIs) for people [24].

2. Sources of Contaminants from the Environment to Food and Water

It is useful to consider the sources of contaminants in order to understand their pathway into food and water sources for consumption. Factors such as soil properties, activities by people, and point sources affect the accumulation of metals in the environment. For example, mining may result in release of substances such as arsenic and mercury [25, 26]. Once in the environment, these substances may contaminate food and water and result in human health hazards, with toxic effects varying depending on the contaminant(s) ingested (Table 1).

2.1. Metals and Metalloids. Metals and metalloids in the environment have various sources. One source of mercury and lead is artisanal gold mining. For example, in the gold mining area of Tongguan, Shaanxi, China, concentrations of these metals in locally produced grains and vegetables exceeded governmental tolerance limits and posed a potential health risk to people from consumption [58]. Lead and cadmium from an iron mine in Morocco resulted in concentrations of cadmium in livestock organs higher than acceptable limits [59]. Likewise, in Spain, sheep near a mine were found to have lead contamination, with levels in 87.5% liver samples above European Union Maximum Residue Levels (MRL) [60].

Table 1: Possible human health hazards due to exposure to food contaminants.

Food contaminants	Possible hazards	References
	Metals/metalloids	
Lead	Complications in the nervous system and red blood cells	[27]
	Reduction in cognitive development and intellectual performance	[28]
	Death among children	[29]
Cadmium	Renal tubular dysfunction, associated with high risk of lung and breast cancer	[30]
	Osteomalacia and osteoporosis	
Arsenic	Associated with dermal, respiratory, nervous, mutagenic, and carcinogenic effects	[31]
Nickel	Associated with dermatotoxicity, lower body weight, and fetotoxicity among pregnant women	[32]
Mercury	Linked to cardiovascular, reproductive, and developmental toxicity, neurotoxicity, nephrotoxicity, immunotoxicity, and carcinogenicity	[33]
	Mycotoxins	
Aflatoxin	Immunodeficiency	[34]
	Aflatoxicosis	[35]
	Primary hepatocellular carcinoma	[36]
	Liver cirrhosis	[37]
Ochratoxin	Nephropathy	[38]
Deoxynivalenol	Impaired intestinal integrity	[39]
	Impaired gut-associated immune system	
Zearalenone	Hyperestrogenism and reproductive dysfunction	[40]
Fumonisins	Esophageal cancer and birth defects	[41]
	Antimicrobials	
Tetracyclines	Impaired intestinal flora	[42]
Quinolones	Drug-resistant pathogens	[43]
Macrolides	Hypersensitivity and anaphylactic shock	[44]
Sulfonamides	Kidney damage and nephropathy	[45]
	Polycyclic aromatic hydrocarbons (PAHs)	
Benzo[a]pyrene	Mutagenicity and carcinogenicity	[46]
	DNA damage and oxidative stress	[47]
	Impaired male fertility	[48]
	Respiratory diseases	[49]
	Cognitive dysfunction among children	[50]
	Pesticides	
Chlorpyrifos	Neurological symptoms	[51]
DDTs	Neurological symptoms	[52]
	Endocrine disruption	[53]
DDT and other OCPs	Infertility and fetal malformation	[54]
	Dioxins and polychlorinated biphenyls	
Dioxins and PCBs	Language delay	[55]
	Disturbances in mental and motor development	[56]
PCBs	Neurological disorders	[57]

Industrial regions often have extensive environmental contamination by metals. In Romania, lead, cadmium, copper, and zinc contaminated crops, exceeding maximum acceptable levels in some samples [61]. In China, cadmium from a zinc smelter contaminated leaf and root vegetables particularly [62]. Arsenic, selenium, lead, and other metal and metalloid contaminants were found near a coking plant in China, contaminating soil and food, and detected in blood samples from children [63]. In that case, ingestion of food was determined to be the major exposure pathway for local children. In Belgium, cadmium was detected in locally produced food items grown near nonferrous metal plants

[64]. Thallium from a steel plant in south China was found to contaminate soil and thence vegetables, exceeding German standards for the maximum permissible level and showing hyperaccumulation in plants such as leaf lettuce, chard, and pak choi [65].

Many fruits and vegetables have been shown to be contaminated by metals. For example, cadmium in soil was detected in navel oranges in China and lead and cadmium in soybeans in Argentina [66, 67]. Also in China, various metals were detected in edible seeds, with levels of copper sufficiently high to show an increased health risk to people consuming them [68]. On the contrary, contamination levels of mercury in rice samples from a city in eastern China were below levels likely to affect human health [69]. In the global arena, methylmercury has been detected in fish and other seafood around the world [70, 71]. Fish tissues from Turkey were shown to be contaminated with copper, iron, zinc, and manganese [72]. Various metals have also been detected in fish from Sicily, with some concentrations exceeding European regulation limits [73]. In Asia, food species of turtles have been shown to contain mercury [74].

With regard to water, endemic arsenism from contaminated drinking water has been reported in China [11]. Monitoring has detected nickel in drinking water in New South Wales, Australia, but levels do not appear to pose any health risk for the local population [75].

Further evidence of potential health risks to people from metals are surveys of human samples. Mercury and monomethylmercury were detected in human hair samples from French Guiana, associated with a diet rich in fish, with 57% of people tested having mercury levels higher than the WHO safety limit [70]. In Spain, mercury, lead, and cadmium have also been detected in human milk samples, with increased levels of lead associated with higher consumption of potatoes [76].

2.2. Polycyclic Aromatic Hydrocarbons. Polycyclic aromatic hydrocarbons (PAHs) primarily occur after organic matter undergoes incomplete combustion or pyrolysis, or from industrial processes [77]. Food contamination comes from the environment, industry, or home cooking (such as when using biomass fuels). These compounds appear to be genotoxic and carcinogenic. Oil spills from transporter ships in the ocean are all too common and will result in contamination of seafood. Besides the petroleum-related polycyclic aromatic hydrocarbon (PAH) compounds, chemical dispersants are often used to mitigate effects of oil in the ocean. After the BP Deepwater Horizon oil spill in Louisiana, USA, in 2010, the Federal government responded to seafood safety concerns by instigating protocols for sampling and analysis of food to determine its safety [78]. Lessons learned after this scenario included recognition of the need to improve risk assessments to adequately protect vulnerable populations, including pregnant women [79].

2.3. Industrial Chemicals. Persistent organic pollutants (POPs) are synthetic organic chemicals; some are used in industry, some as pesticides, and some are by-products from industry or combustion. They include pesticides like aldrin, chlordane and DDT, industrial chemicals like PCBs and HCBs, and unintended by-products like dibenzodioxins and dibenzofurans. They persist in the environment, are distributed globally in air and ocean currents, and accumulate in animals in the food chain (including in humans). Their side effects depend on the chemical and the contaminated species; for example, they may have effects on reproductive or immune systems, or increase cancer risks [80].

Chlorpyrifos is an organophosphate pesticide that affects vision and causes other neurological toxic effects in humans [81]. It has been detected in dietary samples, and foods have been shown to be responsible for approximately 13% of daily exposure to this chemical [51]. Organochlorine pesticides such as DDT have been used in agriculture and vector-transmitted disease control for decades, though their use now is restricted due to known persistence in the environment and toxic effects such as neurological dysfunction and endocrine disruption [82]. Pyrethroids such as permethrin and deltamethrin are widely used for control of vector insects and aircraft disinfection, as they are relatively safe for people [83]. However, their use near foods can result in contamination and studies are ongoing to reduce potential toxic effects [84, 85]. Although neonicotinoids are widespread in the environment and contaminate consumable items, their toxic effects are still not yet well understood [86, 87].

Polychlorinated biphenyls (PCBs) have a variety of uses in industry, including in transformers, as heat exchange fluids or paint additives, or in plastics. Ingestion of PCB residue-contaminated food—especially meat, fish, and poultry—is the main source for people, with ready absorption from the gastrointestinal tract [88, 89]. Contaminated breast milk is a potential source for nursing infants. Chloracne is reported after extensive exposure to PCBs, but immune and carcinogenic effects may also result.

Polybrominated and polychlorinated compounds may originate from anthropogenic and natural sources. They have many uses such as flame retardants and dielectric/coolant fluids in electrical apparatus. Toxic effects include endocrine disruption, neurotoxicity, and cancer. Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) have been detected in human milk in China [90]. This is a particular concern due to the high susceptibility of nursing infants to toxic effects. According to a study in Germany, dietary exposure is the most significant pathway for PBDEs in people [91]. In particular, seafood has been cited as a major contributor [92].

Perfluorinated compounds (PFCs) are synthetic chemicals with friction-resistant properties that make them useful in many materials and industries. Toxic effects include endocrine and immune system disruption and developmental problems. Some precursors or metabolic intermediates for perfluoroalkyl and polyfluoroalkyl substances (PFASs) are toxic, for example, estrogen-like activities [93]. The PFAS group includes perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA); these have been detected in many food sources including seafood in China and Germany [94, 95]. Drinking water and food are the main sources of

exposure to PFOS and PFOA, although levels are usually low [96].

Acrylamide occurs in many foods—generally associated with high heat cooking processes (e.g., in breads and baked or fried potatoes)—and is also manufactured for commercial and industrial uses (such as in paper and dye production, in wastewater treatment, and as a chemical grouting agent) [97]. The IARC has classified acrylamide as a probable human carcinogen, placed in group 2A since 1994 [98].

2.4. Pharmaceuticals and Personal Care Products. The term pharmaceuticals and personal care products (PPCPs) includes a wide range of substances that may enter the environment and thence food or water sources. Antimicrobials and other drugs may originate from use in both humans and animals. For example, swine waste containing antimicrobials may contaminate both water and food [99]. Aside from the very real threat of increased antimicrobial resistance through exposure to extraneous sources of these chemicals, it has also been shown that many drugs have other side effects including endocrine disruption [100]. In some circumstances, the medicinal products themselves may be contaminated, for example, in many herbal products [101].

2.5. Radioactive Elements. Most radioactive elements did not exist naturally, and soil contamination with such material has only become a problem since nuclear weapons and reactors have been developed [102].

After tsunami damage affected the Fukushima nuclear plant in Japan in 2011, monitoring of food and water samples detected contamination above provisional regulation values and restrictions were put in place [103]. Radionucleotides have also been detected in seafood in India, various foods in the Balkans, and food and drinking water in Switzerland [104–106]. Risk assessments are conducted to ensure that levels remain within acceptable limits. Furthermore, experimental models are undertaken to assess safety in ingestion pathways, considering several different food intakes [107]. In the US, there is an FDA rule pertaining to uranium, radium, alpha particle, beta particle, and photon radioactivity in bottled water [108].

2.6. Electronic Waste. Modern society has become encumbered with many electrical devices, and electronic waste (or e-waste) has become a major problem. Inappropriate processing, for example, incomplete combustion, of such products releases a variety of pollutants covered above, including PBDEs, dioxins/furans (PCDD/Fs), PAHs, PCBs, and metals/metalloids [109]. In addition, contamination from such devices can enter drinking water and food [110].

2.7. Plastics. In recent times, we rely more and more on packaging materials—in particular plastics—to transport and help preserve food. These materials are not inert and may themselves contaminate food and drinks as multiple chemicals are released into foods and beverages from food contact materials. These are termed "migrants" and include such chemicals as phthalate plasticizers which have been detected in bottled water [111]. Factors such as higher storage temperatures and prolonged contact time with the packaging were linked to higher levels of contamination, but a health risk assessment showed that the risk for consumers was low [111].

2.8. Nanoparticles. Another recent development is that of nanoparticles. These have one dimension less than 1×10^{-7} m, and engineered nanoparticles have been used in a wide range of products, such as paints, cosmetics, and pesticides [102]. Pathways and effects of these in biota are as yet unclear, but they have been shown to travel in the food chain [112]. Nanosized materials have been detected in foods such as wheat-based products [113].

3. Risk Assessment and Monitoring

As shown in Table 1, each of the possible contaminants in food can be linked to a variety of toxic effects. Any adverse effects seen depend on multiple factors, including whether exposure is acute or chronic, the dose received, the route of exposure, and details of the individual person such as age and health. As an example, lead toxicity affects almost all organs, but the most severely affected is the nervous system [114]. In adults, long-term exposure results in reduced cognitive performance. More severe signs such as learning difficulties and behavioural problems are seen in infants and young children as they are more sensitive during this phase of neurodevelopment [115]. High levels of contamination with lead may also cause kidney damage; chronic exposure may cause anaemia and hypertension, and reduced fertility in males [116]. In pregnant women, high blood lead levels are associated with premature birth or babies with a low birth weight, and this risk is increased in emaciated women [117]. On an individual level, blood sampling is a quick and easy method of assessing circulating levels of lead and can be used to indicate recent or current exposure. However, this does not account for lead stored elsewhere in the body, particularly in bones. X-ray fluorescence can measure whole-body lead in bones, and x-rays may show lead-containing foreign materials [118–120]. Treatment of clinical cases is by using chelating agents, which will reduce blood lead levels, yet neurological effects may remain [121].

On the other hand, at a community level, it may be more important to identify contaminated sites and assess health risks to the general population and thereafter aim to reduce or remove exposure to contaminants such as lead. Thus, monitoring plays a vital role in food safety. Such monitoring has identified contamination of many foods (examples are shown in Table 2). In order to monitor effectively, samples should be analysed from a variety of sources: human samples to detect levels after exposure, diverse foods from the total diet and drinking water sources, and also the environment itself (to identify the source of food contamination). Samples from people frequently include blood, urine, feces, breast milk, hair, and/or semen [122]. Human biomonitoring is notably useful to facilitate risk assessment. A combination of environmental monitoring and biomonitoring may identify

Table 2: Examples of food contamination with different chemicals around the world.

Food contaminants	Foods	Country	References
	Metals/metalloids		
Pb, Hg	Grains and vegetables	China	[58, 62, 67]
Pb, Cd	Livestock organs	Morocco	[59]
Pb	Sheep livers	Spain	[60]
Pb, Cd, Cu, Zn	Agricultural crops	Romania	[61]
Cd	Locally produced foods	Belgium	[64]
Tl	Lettuce and chard	Germany	[65]
Pb, Cd	Soybeans	Argentina	[66]
Cu, Zn, Mn, Fe	Fish	Turkey	[72]
	Mycotoxins		
Deoxynivalenol, zearalenone, T2 toxin and HT-2 toxin	Wheat, barley, Japanese retail foods	Japan	[126]
Aflatoxin, ochratoxin	Wheat flour	China	[127]
Fumonisins	Maize	South Africa	[128]
Nivalenol	Cereals and cereal products	Tunisia	[129]
Aflatoxins	Ground nut oil	Sudan	[130]
	Antimicrobials		
Antimicrobials	Pork meat	Madagascar	[131]
	Table eggs	Sudan	[132]
	Milk	Peru	[133]
	Beef	Nigeria	[134]
	Meat	Brazil	[135]
	Polycyclic aromatic hydrocarbons (PAHs)		
Benzo[a]pyrene Chrysene	Barbecued foods	Sweden	[136]
Anthracene Fluoranthene	Yogurt	Italy	[137]
19 PAHs	Grains, flour, and bran	Poland	[138]
Total PAHs	Oyster	Japan	[139]
	Pesticides and polychlorinated biphenyls (PCBs)		
Chlorpyrifos	Catfish	Australia	[140]
	Vegetables	China	[141]
	Food plant	Algeria	[142]
DDTs and other OCPs	Edible offal	Egypt	[143, 144]
	Milk		
	Chicken products	South Africa	[145]
	Milk	Ethiopia	[146]
	Fish	Mozambique	[6]
PCBs and OCPs	Baby foods	Korea	[147]
OCPs and pyrethroids	Honey	Egypt	[148]
PCBs and OCPs	Cereals	Poland	[149]
PCBs and OCPs	Milk, yak muscle and liver	Tibet Plateau	[150]
	Radioactive substances		
Radioactive substances	Water and food	Japan	[103]
Radionuclides	Seafood	India	[104]
Uranium isotopes	Food	Balkans	[105]
Radioactive substances	Water and food	Switzerland	[106]

risk factors, such as detection of higher levels of cadmium in umbilical cord blood from mothers consuming more than two portions of fish each week [123]. In the case of metals, environmental sampling has shown hotspots of contamination around mining (such as gold, lead, and zinc), electronic waste sites, and industrial areas [110, 124, 125]. Contamination in soils at these sites has been linked to bioaccumulation in agricultural crops and associated increase in human health risk.

Examples of indirect monitoring methods for contaminants in the environment and food include measurement of biomarkers such as proteomics in oysters contaminated with mercury, transcriptome effects in the hepatopancreas of clams, or mutagenicity of seawater in seafood farms associated with PAHs and PCBs [151–153]. High throughput and ultrasensitive screening using nanoparticles has also been utilized for detection of environmental pollutants [154]. Moving forward, testing of chemicals to evaluate potential toxicities before registration and authorised use in the environment may employ tools and concepts such as biomonitoring equivalents and threshold of toxicologic concern, alongside generic and physiologically-based toxicokinetic models [155]. Since 2006, the European Commission has implemented new legislation, called REACH (EC 1907/2006), to identify properties—including toxicities—of chemicals and thus better protect human health and the environment [156]. Other similar legislation exists elsewhere in the world; for example, the Environmental Protection Agency runs a registration process for pesticides to comply with federal laws in the US [157].

Once sources of contaminants have been identified, it is vital to minimize contamination of food. For this purpose, regulations are in place at both national and international levels to restrict contaminated food entering the human food chain. In some cases, legislation exists to assess levels of food contamination. For example, the Marine Strategy Framework Directive in Spain monitors for contaminants in edible tissues of seafood destined for human consumption, assessing levels against established EU standards for food safety [158]. The German Federal Environment Agency monitors both the environment—using the German Environmental Survey (GerES)—and human biomonitoring—using the German Environmental Specimen Bank (ESB) [159]. Amongst others, these have, respectively, been used to detect lead in drinking water and exposure to phthalates and bisphenol A. National monitoring systems may cooperate at an international level. To maintain and improve food safety globally, the Codex Alimentarius contains a set of international food standards, guidelines, and codes of practice [20]. These are based on science from risk assessment bodies or organized by consultations with FAO and WHO. These are voluntary but often form the basis of national legislation.

Food standards and legislation focus on individual food products. To understand the combined risk that someone has from one or many chemicals, a complete dietary risk assessment can be conducted to assess the total potential risk of a typical diet. For example, Zhou et al. assessed the levels of organochlorine pesticides (OCPs) in a total diet from China [160]. The study found that aquatic foods, meats, and cereals were the major foods contributing to contamination of the diet with these chemicals. Multilevel risk assessment can also be used to identify critical points in contamination sources. For example, a study of metals in soil and food in Taiwan identified more than 600 metal-contaminated sites over a period of two decades which could then be targeted for remediation efforts [161].

4. Remediation

Once sources of contamination have been identified, it is possible to consider how best to improve food safety through various methods. Methods of remediation vary depending on the type of contaminants present and in which environment. These can be expensive on a large scale. Remediation may focus on reducing contaminants in the environment overall or reducing concentrations in foods specifically.

A common method used to reduce environmental exposure to contaminants is soil remediation. One simple method is to remove contaminated topsoil, which typically contains higher levels of contaminants than subsoil, from agricultural areas [161]. Alternatively, soil turnover and mixing *in situ* may be sufficient to dilute contaminant, such as metals, concentrations to an acceptable level. Thermal treatment or landfill can also be used to remediate a site. Different soil properties can affect contaminant levels. For example, metal (cadmium, mercury, and chromium) accumulation in flowering Chinese cabbage was shown to be controlled by total metal concentrations in soil and available calcium [162]. It is well known that soil science can be used to improve food quality and quantity [163]. It can similarly be used to reduce contamination of crops. The predominant congener of technical DDT, *p,p*'-DDT, is susceptible to microbial metabolism and rarely accumulates in aerobic soils [164]. Long-term gardening has been shown to result in lower levels of PAHs, possibly due to PAH degradation by enhanced microbial activity, and/or dilution [9]. Microbial bioremediation may also be used to reduce levels of metal contamination of soils in an environmentally-friendly manner [165].

Different forms of phytoremediation may be used to either remove contamination from soils or to reduce contamination of plants. If the plants are crops for consumption, reduced uptake is beneficial. One example of phytoremediation is selection of plants to specifically remove contaminants from agricultural land, such as using black nightshade (*Solanum nigrum* L.) for removal of thallium from soil [166]. A study by Yu et al. on cadmium-contaminated agricultural land showed differential accumulation of cadmium in two oilseed rape cultivars [167]. Interestingly, the study also showed increased uptake of cadmium in rice crops planted after the oilseed rape harvest, with contamination of rice higher compared to a crop after a fallow period. Another mechanism of plants which can be used advantageously is that of reduced accumulation of unwanted chemicals in certain cultivars or altered plant hybrids—for example in Chinese kale—and these can be selected to produce safer food [168].

Crop management techniques can affect contamination of plants. Use of slow-release nitrogen fertilizers can reduce cadmium levels in plants such as pak choi, as the plants

appear to have stronger tolerance to the metal and a lower efficiency of translocation to edible plant parts compared to those grown using typical fertilizers [169]. Contaminated water used to flood paddy fields is a huge problem in countries that rely on rice crops. Water management—such as drying the paddy field for a period of days between late tillering and young ear differentiation stages—has been shown to reduce cadmium and arsenic levels in rice crops of different rice species [170]. Human health risks from medicinal products contaminating food and water for consumption may be modelled, for example, using pond aquaculture, to identify potential health risks [171].

Exposure to contaminants on foods prepared for consumption can also be reduced by using safer storage alternatives, such as edible films and coatings [172]. Contamination of foods with PAHs from cooking can be greatly reduced by avoiding smoking or open fires but rather replacing them with gas stoves for cooking [77]. Several nongovernmental organizations and charities offer gas stoves to families to help alleviate this source of food contamination, which is a risk particularly for women and children who spend more time at home [173].

5. Summary and Conclusions

Attitudes in society towards food safety and contamination are often rooted in tradition and habit. Although consumers select their diet based on social and financial factors, the remit for food safety remains firmly with regulatory bodies. These bodies can monitor for contaminants and enforce legislation. Aspects of food contamination also have political implications. As mentioned above, food safety laws are necessary, with monitoring of food and water contamination, as well as enacting measures to reduce and eliminate exposure to environmental pollutants. Publicity after environmental pollution-related incidents behoves a government to have public health, legal, and ethical frameworks in place in a timely manner. Education of society regarding safer crop cultivation and livestock rearing, selection of a balanced diet, and safer cooking methods should also be encouraged. On a nationwide scale, governments also should endeavour to reduce urban disparities in environmental exposures. Although some contaminants have focal effects, many are transported globally. For this reason, an international stance on food safety is necessary by reducing environmental and food contamination and ensuring trade of safer food products on a global scale.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

This work was supported by the Faculty of Veterinary Medicine, Hokkaido University.

References

[1] S. Stasinos, C. Nasopoulou, C. Tsikrika, and I. Zabetakis, "The bioaccumulation and physiological effects of heavy metals in carrots, onions, and potatoes and dietary implications for Cr and Ni: a review," *Journal of Food Science*, vol. 79, no. 5, pp. R765–R780, 2014.

[2] P. Price, R. Zaleski, H. Hollnagel, H. Ketelslegers, and X. Han, "Assessing the safety of co-exposure to food packaging migrants in food and water using the maximum cumulative ratio and an established decision tree," *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, vol. 31, no. 3, pp. 414–421, 2014.

[3] T. Leibson, P. Lala, and S. Ito, "Drug and Chemical Contaminants in Breast Milk: Effects on Neurodevelopment of the Nursing Infant," in *Handbook of Developmental Neurotoxicology*, W. J. Slikker, M. G. Paule, and., and C. Wang, Eds., pp. 275–284, Elsevier, 2018.

[4] M. Vafeiadi, V. Georgiou, G. Chalkiadaki et al., "Association of prenatal exposure to persistent organic pollutants with obesity and cardiometabolic traits in early childhood: the Rhea mother-child cohort (Crete, Greece)," *Environmental Health Perspectives*, vol. 123, no. 10, 2015.

[5] A. Mahmood and R. N. Malik, "Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan," *Arabian Journal of Chemistry*, vol. 7, pp. 91–99, 2014.

[6] L. A. Thompson, Y. Ikenaka, Y. B. Yohannes et al., "Human Health Risk from Consumption of Marine Fish Contaminated with DDT and Its Metabolites in Maputo Bay, Mozambique," *Bulletin of Environmental Contamination and Toxicology*, pp. 1–5, 2018.

[7] C. F. da Araújo, M. V. Lopes, M. R. Vasquez et al., "Cadmium and lead in seafood from the Aratu Bay, Brazil and the human health risk assessment," *Environmental Modeling & Assessment*, vol. 188, no. 4, 2016.

[8] H. Kohrman and C. P. Chamberlain, "Heavy metals in produce from urban farms in the San Francisco Bay Area," *Food Additives & Contaminants: Part B. Surveillance*, vol. 7, no. 2, pp. 127–134, 2014.

[9] M. L. Kaiser, M. L. Williams, N. Basta, M. Hand, and S. Huber, "When vacant lots become urban gardens: Characterizing the perceived and actual food safety concerns of urban agriculture in Ohio," *Journal of Food Protection*, vol. 78, no. 11, pp. 2070–2080, 2015.

[10] J. Enault, S. Robert, O. Schlosser, C. de Thé, and J.-F. Loret, "Drinking water, diet, indoor air: Comparison of the contribution to environmental micropollutants exposure," *International Journal of Hygiene and Environmental Health*, vol. 218, no. 8, pp. 723–730, 2015.

[11] Y. Jin, C. Liang, G. He, and J. Cao, "Study on distribution of endemic arsenism in China," *Wei sheng yan jiu = Journal of hygiene research*, vol. 32, no. 6, pp. 519–540, 2003.

[12] R. J. B. Peters, H. Beeltje, and R. J. Van Delft, "Xeno-estrogenic compounds in precipitation," *Journal of Environmental Monitoring*, vol. 10, no. 6, pp. 760–769, 2008.

[13] S. E. Nelms, T. S. Galloway, B. J. Godley, D. S. Jarvis, and P. K. Lindeque, "Investigating microplastic trophic transfer in marine top predators," *Environmental Pollution*, 2018.

[14] R. T. Enander, H. J. Cohen, D. M. Gute, L. C. Brown, A. M. C. Desmaris, and R. Missaghian, "Lead and methylene chloride exposures among automotive repair technicians," *Journal of*

Occupational and Environmental Hygiene, vol. 1, no. 2, pp. 119–125, 2004.

[15] L. Järup, "Hazards of heavy metal contamination," *British Medical Bulletin*, vol. 68, no. 1, pp. 167–182, 2003.

[16] Y. Lu, S. Song, R. Wang et al., "Impacts of soil and water pollution on food safety and health risks in China," *Environment International*, vol. 77, pp. 5–15, 2015.

[17] Q. Zhao, Y. Wang, Y. Cao et al., "Potential health risks of heavy metals in cultivated topsoil and grain, including correlations with human primary liver, lung and gastric cancer, in Anhui province, Eastern China," *Science of the Total Environment*, vol. 470-471, pp. 340–347, 2014.

[18] D. Jonker, A. Freidig, J. Groten et al., "Safety Evaluation of Chemical Mixtures and Combinations of Chemical and Non-Chemical Stressors," *Reviews on Environmental Health*, vol. 19, no. 2, pp. 83–140, 2004.

[19] The Stockholm Convention, "Status of Ratification," http://chm.pops.int/Countries/StatusofRatifications/PartiesandSignatoires/tabid/4500/Default.aspx.

[20] "FAO/WHO Codex Alimentarius Commission," *Food and Cosmetics Toxicology*, vol. 4, p. 601, 1966.

[21] JECFA, "80th Joint FAO/WHO Expert Committee on Food Additives (JECFA) meeting - Food additives and contaminants," *Summary and Conclusions*, vol. 71, pp. 1–16, 2015.

[22] JECFA, "Safety evaluation of certain food additives and contaminants," 2011.

[23] Codex Alimentarius Commssion, Pesticide Residues 50th session in Haikou China.

[24] JMPR, AGP - JMPR Reports and evaluations.

[25] P. L. Smedley and D. G. Kinniburgh, "A review of the source, behaviour and distribution of arsenic in natural waters," *Applied Geochemistry*, vol. 17, no. 5, pp. 517–568, 2002.

[26] N. Bortey-Sam, S. M. M. Nakayama, Y. Ikenaka et al., "Human health risks from metals and metalloid via consumption of food animals near gold mines in Tarkwa, Ghana: Estimation of the daily intakes and target hazard quotients (THQs)," *Ecotoxicology and Environmental Safety*, vol. 111, pp. 160–167, 2015.

[27] W. S. Darwish, A. S. Atia, M. H. Khedr, and W. F. Eldin, "Metal contamination in quail meat: residues, sources, molecular biomarkers, and human health risk assessment," *Environmental Science and Pollution Research*, vol. 25, no. 20, pp. 20106–20115, 2018.

[28] M. Szkup-Jabłońska, B. Karakiewicz, E. Grochans et al., "Effects of blood lead and cadmium levels on the functioning of children with behaviour disorders in the family environment," *Annals of Agricultural and Environmental Medicine*, vol. 19, no. 2, pp. 241–246, 2012.

[29] J. Yabe, S. M. M. Nakayama, Y. Ikenaka et al., "Lead poisoning in children from townships in the vicinity of a lead-zinc mine in Kabwe, Zambia," *Chemosphere*, vol. 119, pp. 941–947, 2015.

[30] G. F. Nordberg, K. Nogawa, M. Nordberg, and L. T. Friberg, "Cadmium," *Handbook on the Toxicology of Metals*, pp. 445–486, 2007.

[31] H.-J. Lin, T.-I. Sung, C.-Y. Chen, and H.-R. Guo, "Arsenic levels in drinking water and mortality of liver cancer in Taiwan," *Journal of Hazardous Materials*, vol. 262, pp. 1132–1138, 2013.

[32] United States Environmental Protection Agency (US EPA), *Quantification of toxicologic effects for nickel. Prepared by the office of health and environmental assessment, environmental criteria and assessment office, Cincinnati, OH for the office of water, office of science and technology*, Washington, DC, USA, 1991.

[33] G. Genchi, M. S. Sinicropi, A. Carocci, G. Lauria, and A. Catalano, "Mercury exposure and heart diseases," *International Journal of Environmental Research and Public Health*, vol. 14, no. 1, 2017.

[34] W. S. Darwish, Y. Ikenaka, S. M. M. Nakayama, and M. Ishizuka, "An overview on mycotoxin contamination of foods in Africa," *Journal of Veterinary Medical Science*, vol. 76, no. 6, pp. 789–797, 2014.

[35] E. Azziz-Baumgartner, K. Lindblade, and K. Gieseker et al., "Case–Control Study of an Acute Aflatoxicosis Outbreak, Kenya, 2004," *Environmental Health Perspectives*, vol. 113, no. 12, pp. 1779–1783, 2005.

[36] P. C. Turner, C. Loffredo, S. E. Kafrawy et al., "Pilot survey of aflatoxin–albumin adducts in sera from Egypt," *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, vol. 25, no. 5, pp. 583–587, 2008.

[37] M. H. Kuniholm, O. A. Lesi, M. Mendy et al., "Aflatoxin exposure and viral hepatitis in the etiology of liver cirrhosis in The Gambia, West Africa," *Environmental Health Perspectives*, vol. 116, no. 11, pp. 1553–1557, 2008.

[38] K. Hmaissia Khlifa, R. Ghali, C. Mazigh, Z. Aouni, S. Machgoul, and A. Hedhili, "Ochratoxin A levels in human serum and foods from nephropathy patients in Tunisia: Where are you now?" *Experimental and Toxicologic Pathology*, vol. 64, no. 5, pp. 509–512, 2012.

[39] H. Robert, D. Payros, P. Pinton, V. Théodorou, M. Mercier-Bonin, and I. P. Oswald, "Impact of mycotoxins on the intestine: are mucus and microbiota new targets?" *Journal of Toxicology and Environmental Health - Part B: Critical Reviews*, vol. 20, no. 5, pp. 249–275, 2017.

[40] H. Chang, W. Kim, J. Park et al., "The Occurrence of Zearalenone in South Korean Feedstuffs between 2009 and 2016," *Toxins*, vol. 9, no. 7, p. 223, 2017.

[41] J. Park, H. Chang, S. Hong, D. Kim, S. Chung, and C. Lee, "A decrease of incidence cases of fumonisins in south Korean feedstuff between 2011 and 2016," *Toxins*, vol. 9, no. 9, 2017.

[42] W. S. Darwish, E. Eldaly, M. El-Abbasy, Y. Ikenaka, and M. Ishizuka, "Antibiotic residues in food: African scenario," *Japanese Journal of Veterinary Research2*, vol. 61, pp. S13–S22, 2013.

[43] S. A. E. Kools, J. F. Moltmann, and T. Knacker, "Estimating the use of veterinary medicines in the European Union," *Regulatory Toxicology and Pharmacology*, vol. 50, no. 1, pp. 59–65, 2008.

[44] R. M. El Bayomi, W. S. Darwish, A. M. A. El-Moaty, and T. M. Gad, "Prevalence, antibiogram, molecular characterization and reduction trial of Salmonella typhimurium isolated from different fish species," *Japanese Journal of Veterinary Research*, vol. 64, pp. S181–S186, 2016.

[45] W. S. Darwish, A. S. Atia, L. M. Reda, A. E. Elhelaly, L. A. Thompson, and W. F. Saad Eldin, "Chicken giblets and wastewater samples as possible sources of methicillin-resistant Staphylococcus aureus: Prevalence, enterotoxin production, and antibiotic susceptibility," *Journal of Food Safety*, vol. 38, no. 4, 2018.

[46] W. S. Darwish, Y. Ikenaka, S. Nakayama, H. Mizukawa, and M. Ishizuka, "Mutagenicity of modelled-heat-treated meat extracts: Mutagenicity assay, analysis and mechanism of mutagenesis," *Japanese Journal of Veterinary Research*, vol. 63, no. 4, pp. 173–182, 2015.

[47] W. S. Darwish, Y. Ikenaka, S. Nakayama, H. Mizukawa, L. A. Thompson, and M. Ishizuka, "β-carotene and retinol reduce benzo[a]pyrene-induced mutagenicity and oxidative stress via transcriptional modulation of xenobiotic metabolizing enzymes in human HepG2 cell line," *Environmental Science and Pollution Research*, vol. 25, no. 7, pp. 6320–6328, 2018.

[48] J. B. Dai, Z. X. Wang, and Z. D. Qiao, "The hazardous effects of tobacco smoking on male fertility," *Asian Journal of Andrology*, 2015.

[49] M. Jumpponen, H. Rönkkömäki, P. Pasanen, and J. Laitinen, "Occupational exposure to gases, polycyclic aromatic hydrocarbons and volatile organic compounds in biomass-fired power plants," *Chemosphere*, vol. 90, no. 3, pp. 1289–1293, 2013.

[50] W. A. Jedrychowski, F. P. Perera, D. Camann et al., "Prenatal exposure to polycyclic aromatic hydrocarbons and cognitive dysfunction in children," *Environmental Science and Pollution Research*, vol. 22, no. 5, pp. 3631–3639, 2015.

[51] Y. Pang, D. L. MacIntosh, D. E. Camann, and P. B. Ryan, "Analysis of aggregate exposure to chlorpyrifos in the NHEXAS-Maryland investigation," *Environmental Health Perspectives*, vol. 110, no. 3, pp. 235–240, 2002.

[52] L. A. Thompson, W. S. Darwish, Y. Ikenaka, S. M. M. Nakayama, H. Mizukawa, and M. Ishizuka, "Organochlorine pesticide contamination of foods in Africa: Incidence and public health significance," *Journal of Veterinary Medical Science*, vol. 79, no. 4, pp. 751–764, 2017.

[53] G. C. Windham, D. Lee, P. Mitchell, M. Anderson, M. Petreas, and B. Lasley, "Exposure to organochlorine compounds and effects on ovarian function," *Epidemiology*, vol. 16, no. 2, pp. 182–190, 2005.

[54] R. W. Bretveld, M. Hooiveld, G. A. Zielhuis, A. Pellegrino, I. A. L. M. van Rooij, and N. Roeleveld, "Reproductive disorders among male and female greenhouse workers," *Reproductive Toxicology*, vol. 25, no. 1, pp. 107–114, 2008.

[55] I. H. Caspersen, M. Haugen, S. Schjølberg et al., "Maternal dietary exposure to dioxins and polychlorinated biphenyls (PCBs) is associated with language delay in 3 year old Norwegian children," *Environment International*, vol. 91, pp. 180–187, 2016.

[56] S. Nakajima, Y. Saijo, S. Kato et al., "Effects of prenatal exposure to polychlorinated biphenyls and dioxins on mental and motor development in Japanese children at 6 months of age," *Environmental Health Perspectives*, vol. 114, no. 5, pp. 773–778, 2006.

[57] G. Winneke, "Developmental aspects of environmental neurotoxicology: lessons from lead and polychlorinated biphenyls," *Journal of the Neurological Sciences*, vol. 308, no. 1-2, pp. 9–15, 2011.

[58] R. Xiao, S. Wang, R. Li, J. J. Wang, and Z. Zhang, "Soil heavy metal contamination and health risks associated with artisanal gold mining in Tongguan, Shaanxi, China," *Ecotoxicology and Environmental Safety*, vol. 141, pp. 17–24, 2017.

[59] M. Nouri and A. Haddioui, "Human and animal health risk assessment of metal contamination in soil and plants from Ait Ammar abandoned iron mine, Morocco," *Environmental Modeling & Assessment*, vol. 188, no. 1, pp. 1–12, 2016.

[60] J. Pareja-Carrera, R. Mateo, and J. Rodríguez-Estival, "Lead (Pb) in sheep exposed to mining pollution: Implications for animal and human health," *Ecotoxicology and Environmental Safety*, vol. 108, pp. 210–216, 2014.

[61] M. Nedelescu, D. Baconi, A. Neagoe et al., "Environmental metal contamination and health impact assessment in two industrial regions of Romania," *Science of the Total Environment*, vol. 580, pp. 984–995, 2017.

[62] B. Li, Y. Wang, Y. Jiang et al., "The accumulation and health risk of heavy metals in vegetables around a zinc smelter in northeastern China," *Environmental Science and Pollution Research*, vol. 23, no. 24, pp. 25114–25126, 2016.

[63] S. Cao, X. Duan, X. Zhao et al., "Health risks from the exposure of children to As, Se, Pb and other heavy metals near the largest coking plant in China," *Science of the Total Environment*, vol. 472, pp. 1001–1009, 2014.

[64] V. Vromman, C. Saegerman, L. Pussemier et al., "Cadmium in the food chain near non-ferrous metal production sites," *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, vol. 25, no. 3, pp. 293–301, 2008.

[65] J. Liu, X. Luo, J. Wang et al., "Thallium contamination in arable soils and vegetables around a steel plant—A newly-found significant source of Tl pollution in South China," *Environmental Pollution*, vol. 224, pp. 445–453, 2017.

[66] M. J. Salazar, J. H. Rodriguez, G. L. Nieto, and M. L. Pignata, "Effects of heavy metal concentrations (Cd, Zn and Pb) in agricultural soils near different emission sources on quality, accumulation and food safety in soybean [Glycine max (L.) Merrill]," *Journal of Hazardous Materials*, vol. 233-234, pp. 244–253, 2012.

[67] J. Cheng, C. Ding, X. Li, T. Zhang, and X. Wang, "Heavy metals in navel orange orchards of Xinfeng County and their transfer from soils to navel oranges," *Ecotoxicology and Environmental Safety*, vol. 122, pp. 153–158, 2015.

[68] Z.-F. Chen, Y. Zhao, Y. Zhu et al., "Health risks of heavy metals in sewage-irrigated soils and edible seeds in Langfang of Hebei province, China," *Journal of the Science of Food and Agriculture*, vol. 90, no. 2, pp. 314–320, 2010.

[69] G. Wang, Y. Gong, Y.-X. Zhu, A.-J. Miao, L.-Y. Yang, and H. Zhong, "Assessing the risk of Hg exposure associated with rice consumption in a typical city (Suzhou) in eastern China," *International Journal of Environmental Research and Public Health*, vol. 14, no. 5, 2017.

[70] N. Fréry, R. Maury-Brachet, E. Maillot, M. Deheeger, B. De Mérona, and A. Boudou, "Gold-mining activities and mercury contamination of native Amerindian communities in French Guiana: Key role of fish in dietary uptake," *Environmental Health Perspectives*, vol. 109, no. 5, pp. 449–456, 2001.

[71] Q. Peng, B. K. Greenfield, F. Dang, and H. Zhong, "Human exposure to methylmercury from crayfish (Procambarus clarkii) in China," *Environmental Geochemistry and Health*, vol. 38, no. 1, pp. 169–181, 2016.

[72] S. Tekin-Özan, "Determination of heavy metal levels in water, sediment and tissues of tench (*Tinca tinca* L., 1758) from Beyşehir Lake (Turkey)," *Environmental Modeling & Assessment*, vol. 145, no. 1–3, pp. 295–302, 2008.

[73] C. Copat, F. Bella, M. Castaing, R. Fallico, S. Sciacca, and M. Ferrante, "Heavy metals concentrations in fish from Sicily (Mediterranean Sea) and evaluation of possible health risks to consumers," *Bulletin of Environmental Contamination and Toxicology*, vol. 88, no. 1, pp. 78–83, 2012.

[74] A. D. Green, K. A. Buhlmann, C. Hagen, C. Romanek, and J. Whitfield Gibbons, "Mercury contamination in turtles and implications for human health," *Journal of Environmental Health*, vol. 72, no. 10, pp. 14–22, 2010.

[75] N. Alam, S. J. Corbett, and H. C. Ptolemy, "Environmental health risk assessment of nickel contamination of drinking

water in a country town in NSW.," *Public Health Research & Practice* , vol. 19, no. 9-10, pp. 170–173, 2008.

[76] E. García-Esquinas, B. Pérez-Gómez, M. A. Fernández et al., "Mercury, lead and cadmium in human milk in relation to diet, lifestyle habits and sociodemographic variables in Madrid (Spain)," *Chemosphere*, vol. 85, no. 2, pp. 268–276, 2011.

[77] J. Alexander, D. Benford, A. Cockburn et al., "Polycyclic Aromatic Hydrocarbons in Food Scientific Opinion of the Panel on Contaminants in the Food Chain (Question N∘ EFSA-Q-2007-136)," *The European Food Safety Authority Journal*, vol. 724, pp. 1–114, 2008.

[78] G. M. Ylitalo, M. M. Krahn, W. W. Dickhoff et al., "Federal seafood safety response to the Deepwater Horizon oil spill," *Proceedings of the National Acadamy of Sciences of the United States of America*, vol. 109, no. 50, pp. 20274–20279, 2012.

[79] M. Rotkin-Ellman, K. K. Wong, and G. M. Solomon, "Seafood contamination after the BP gulf oil spill and risks to vulnerable populations: A critique of the FDA risk assessment," *Environmental Health Perspectives*, vol. 120, no. 2, pp. 157–161, 2012.

[80] Q. Q. Li, A. Loganath, Y. S. Chong, J. Tan, and J. P. Obbard, "Persistent organic pollutants and adverse health effects in humans," *Journal of Toxicology and Environmental Health, Part A. Current Issues*, vol. 69, no. 21, pp. 1987–2005, 2006.

[81] National Pesticide Information Center, "Chlorpyrifos Technical Fact Sheet," http://npic.orst.edu/factsheets/archive/chlorptech.html.

[82] UNEP, "The Stockholm Convention on Persistent Organic Pollutants, Geneva, Switzerland," 2001, http://chm.pops.int/Portals/0/Repository/convention_text/UNEP-POPS-COP-CONVTEXT-FULL.English.PDF.

[83] WHOPES, "Safety of Pyrethroids for Public Health Use," 2005.

[84] O. Akoto, S. Gavor, M. K. Appah, and J. Apau, "Estimation of human health risk associated with the consumption of pesticide-contaminated vegetables from Kumasi, Ghana," *Environmental Modeling & Assessment*, vol. 187, no. 5, 2015.

[85] M. M. Abdel-Daim, S. M. M. Abuzead, and S. M. Halawa, "Protective role of *Spirulina platensis* against acute deltamethrin-induced toxicity in rats," *PLoS ONE*, vol. 8, no. 9, Article ID e72991, 2013.

[86] A. M. Cimino, A. L. Boyles, K. A. Thayer, and M. J. Perry, "Effects of neonicotinoid pesticide exposure on human health: A systematic review," *Environmental Health Perspectives*, vol. 125, no. 2, pp. 155–162, 2017.

[87] Y. Ikenaka, K. Fujioka, T. Kawakami et al., "Contamination by neonicotinoid insecticides and their metabolites in Sri Lankan black tea leaves and Japanese green tea leaves," *Toxicology Reports*, vol. 5, pp. 744–749, 2018.

[88] World Health Organization, "Polychlorinated biphenyls: human health aspects," http://www.who.int/pcs/pubs/pub_cicad.htm.

[89] Agency for Toxic Substances & Disease Registry, "Toxicological Profile for Polychlorinated Biphenyls (PCBs)," 2000.

[90] L. Zhang, J. Li, Y. Zhao et al., "A national survey of polybrominated diphenyl ethers (PBDEs) and indicator polychlorinated biphenyls (PCBs) in Chinese mothers' milk," *Chemosphere*, vol. 84, no. 5, pp. 625–633, 2011.

[91] H. Fromme, W. Körner, N. Shahin et al., "Human exposure to polybrominated diphenyl ethers (PBDE), as evidenced by data from a duplicate diet study, indoor air, house dust, and biomonitoring in Germany," *Environment International*, vol. 35, no. 8, pp. 1125–1135, 2009.

[92] R. Cruz, S. C. Cunha, and S. Casal, "Brominated flame retardants and seafood safety: A review," *Environment International*, vol. 77, pp. 116–131, 2015.

[93] R. C. Buck, J. Franklin, U. Berger et al., "Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins," *Integrated Environmental Assessment and Management*, vol. 7, no. 4, pp. 513–541, 2011.

[94] Y. Wu, Y. Wang, J. Li et al., "Perfluorinated compounds in seafood from coastal areas in China," *Environment International*, vol. 42, no. 1, pp. 67–71, 2012.

[95] A. Schuetze, T. Heberer, S. Effkemann, and S. Juergensen, "Occurrence and assessment of perfluorinated chemicals in wild fish from Northern Germany," *Chemosphere*, vol. 78, no. 6, pp. 647–652, 2010.

[96] E. D. Van Asselt, J. Kowalczyk, J. C. H. Van Eijkeren et al., "Transfer of perfluorooctane sulfonic acid (PFOS) from contaminated feed to dairy milk," *Food Chemistry*, vol. 141, no. 2, pp. 1489–1495, 2013.

[97] J. Kumar, S. Das, and S. L. Teoh, "Dietary Acrylamide and the Risks of Developing Cancer: Facts to Ponder," *Frontiers in Nutrition*, vol. 5, 2018.

[98] IARC (International Agency for Research on Cancer), "Agents Classified by the IARC Monographs, Volumes 1-123," https://monographs.iarc.fr/wp-content/uploads/2018/09/List_of_Classifications.pdf.

[99] L.-Y. He, G.-G. Ying, Y.-S. Liu et al., "Discharge of swine wastes risks water quality and food safety: Antibiotics and antibiotic resistance genes from swine sources to the receiving environments," *Environment International*, vol. 92-93, pp. 210–219, 2016.

[100] E. R. Kabir, M. S. Rahman, and I. Rahman, "A review on endocrine disruptors and their possible impacts on human health," *Environmental Toxicology and Pharmacology*, vol. 40, no. 1, pp. 241–258, 2015.

[101] P. Posadzki, L. Watson, and E. Ernst, "Contamination and adulteration of herbal medicinal products (HMPs): an overview of systematic reviews," *European Journal of Clinical Pharmacology*, vol. 69, no. 3, pp. 295–307, 2013.

[102] J. S. Walker and G. W. Don, *Mathematics and music*, CRC Press, Boca Raton, FL, 2013.

[103] N. Hamada, H. Ogino, and Y. Fujimichi, "Safety regulations of food and water implemented in the first year following the Fukushima nuclear accident.," *Journal of Radiation Research*, vol. 53, no. 5, pp. 641–671, 2012.

[104] M. F. Khan and S. Godwin Wesley, "Assessment of health safety from ingestion of natural radionuclides in seafoods from a tropical coast, India," *Marine Pollution Bulletin*, vol. 62, no. 2, pp. 399–404, 2011.

[105] F. P. Carvalho and J. M. Oliveira, "Uranium isotopes in the Balkan's environment and foods following the use of depleted uranium in the war," *Environment International*, vol. 36, no. 4, pp. 352–360, 2010.

[106] M. S. Brennwald and F. van Dorp, "Radiological risk assessment and biosphere modelling for radioactive waste disposal in Switzerland," *Journal of Environmental Radioactivity*, vol. 100, no. 12, pp. 1058–1061, 2009.

[107] G. Pröhl, G. Olyslaegers, B. Kanyar et al., "Development and comparison of five site-specific biosphere models for safety assessment of radioactive waste disposal," *Journal of Radiological Protection*, vol. 25, no. 4, pp. 343–373, 2005.

[108] Food and Drug Administration HHS, "Beverages: bottled water. Direct final rule," *Federal register*, vol. 68, no. 41, pp. 9873–9882, 2003.

[109] M. H. Wong, S. C. Wu, W. J. Deng et al., "Export of toxic chemicals - A review of the case of uncontrolled electronic-waste recycling," *Environmental Pollution*, vol. 149, no. 2, pp. 131–140, 2007.

[110] Q. Liu, J. Cao, K. Q. Li et al., "Chromosomal aberrations and DNA damage in human populations exposed to the processing of electronics waste," *Environmental Science and Pollution Research*, vol. 16, no. 3, pp. 329–338, 2009.

[111] M. Zare Jeddi, N. Rastkari, R. Ahmadkhaniha, M. Yunesian, R. Nabizadeh, and R. Daryabeygi, "A margin of exposure approach to assessment of non-cancerous risk of diethyl phthalate based on human exposure from bottled water consumption," *Environmental Science and Pollution Research*, vol. 22, no. 24, pp. 19518–19528, 2015.

[112] J. Kalman, K. B. Paul, F. R. Khan, V. Stone, and T. F. Fernandes, "Characterisation of bioaccumulation dynamics of three differently coated silver nanoparticles and aqueous silver in a simple freshwater food chain," *Environmental Chemistry*, vol. 12, no. 6, pp. 662–672, 2015.

[113] A. M. Gatti, D. Tossini, A. Gambarelli, S. Montanari, and F. Capitani, "Investigation of the presence of inorganic micro- and nanosized contaminants in bread and biscuits by environmental scanning electron microscopy," *Critical Reviews in Food Science and Nutrition*, vol. 49, no. 3, pp. 275–282, 2009.

[114] A. L. Wani, A. Ara, and J. A. Usmani, "Lead toxicity: A review," *Interdisciplinary Toxicology*, vol. 8, no. 2, pp. 55–64, 2015.

[115] R. Rubin and D. S. Strayer, *Environmental and Nutritional pathology. in. Rubins pathology*, Clinicopathologic Foundations of Medicine, 2008.

[116] R. Z. Sokol and N. Berman, "The effect of age of exposure on lead-induced testicular toxicity," *Toxicology*, vol. 69, no. 3, pp. 269–278, 1991.

[117] L. M. Cleveland, M. L. Minter, K. A. Cobb, A. A. Scott, and V. F. German, "Pregnant Women," *American Journal of Nursing*, vol. 108, no. 10, pp. 40–49, 2008.

[118] L. D. Grant, "Environmental toxicants: human exposures and their health effects," in *Wiley-Interscience*, M. Lippman, Ed., pp. 757–809, 2009.

[119] M. J. Kosnett, *Poisoning and Drug Overdose*, K. R. Olson, Ed., McGraw Hill Medical, 2012.

[120] M. J. Kosnett, "Heavy metal intoxication and chelators," in *Basic and Clinical Pharmacology*, B. G. Katzung, Ed., McGraw Hill Professional, 2007.

[121] W. J. Rogan, K. N. Dietrich, J. H. Ware et al., "The effect of chelation therapy with succimer on neuropsychological development in children exposed to lead," *The New England Journal of Medicine*, vol. 344, no. 19, pp. 1421–1426, 2001.

[122] V. Yusa, M. Millet, C. Coscolla, and M. Roca, "Analytical methods for human biomonitoring of pesticides. A review," *Analytica Chimica Acta*, vol. 891, pp. 15–31, 2015.

[123] A. Dursun, K. Yurdakok, S. S. Yalcin et al., "Maternal risk factors associated with lead, mercury and cadmium levels in umbilical cord blood, breast milk and newborn hair," *The Journal of Maternal-Fetal and Neonatal Medicine*, vol. 29, no. 6, pp. 954–961, 2016.

[124] N. Bortey-Sam, S. M. M. Nakayama, O. Akoto et al., "Accumulation of heavy metals and metalloid in foodstuffs from agricultural soils around Tarkwa area in Ghana, and associated human health risks," *International Journal of Environmental Research and Public Health*, vol. 12, no. 8, pp. 8811–8827, 2015.

[125] S. Bose-O'Reilly, J. Yabe, J. Makumba, P. Schutzmeier, B. Ericson, and J. Caravanos, "Lead intoxicated children in Kabwe, Zambia," *Environmental Research*, vol. 165, pp. 420–424, 2018.

[126] T. Yoshinari, H. Takeuchi, K. Aoyama et al., "Occurrence of four Fusarium mycotoxins, deoxynivalenol, zearalenone, T-2 toxin, and HT-2 toxin, in wheat, barley, and Japanese retail food," *Journal of Food Protection*, vol. 77, no. 11, pp. 1940–1946, 2014.

[127] Y. Liu, Y. Lu, L. Wang, F. Chang, and L. Yang, "Survey of 11 mycotoxins in wheat flour in Hebei province, China," *Food Additives & Contaminants: Part B. Surveillance*, vol. 8, no. 4, pp. 250–254, 2015.

[128] H.-M. Burger, M. J. Lombard, G. S. Shephard, J. R. Rheeder, L. van der Westhuizen, and W. C. A. Gelderblom, "Dietary fumonisin exposure in a rural population of South Africa," *Food and Chemical Toxicology*, vol. 48, no. 8-9, pp. 2103–2108, 2010.

[129] A. B. Serrano, G. Font, M. J. Ruiz, and E. Ferrer, "Co-occurrence and risk assessment of mycotoxins in food and diet from Mediterranean area," *Food Chemistry*, vol. 135, no. 2, pp. 423–429, 2012.

[130] S. Z. B. Elshafie, A. ElMubarak, S. A. F. El-Nagerabi, and A. E. Elshafie, "Aflatoxin B1 Contamination of Traditionally Processed Peanuts Butter for Human Consumption in Sudan," *Mycopathologia*, vol. 171, no. 6, pp. 435–439, 2011.

[131] M. Rakotoharinome, D. Pognon, T. Randriamparany et al., "Prevalence of antimicrobial residues in pork meat in Madagascar," *Tropical Animal Health and Production*, vol. 46, no. 1, pp. 49–55, 2014.

[132] M. M. Sirdar, J. Picard, S. Bisschop, A. R. Jambalang, and B. Gummow, "A survey of antimicrobial residues in table eggs in Khartoum State, Sudan, 2007-2008," *Onderstepoort Journal of Veterinary Research*, vol. 79, no. 1, pp. 1–9, 2012.

[133] L. Redding, F. Cubas-Delgado, M. Sammel et al., "Antibiotic residues in milk from small dairy farms in rural Peru," *Food Additives & Contaminants: Part A*, vol. 31, no. 6, pp. 1001–1008, 2014.

[134] G. K. Omeiza, I. E. Ajayi, and O. J. Ode, "Assessment of antimicrobial drug residues in beef inabuja, the federal capital territory, Nigeria," *Veterinaria Italiana*, vol. 48, no. 3, pp. 283–289, 2012.

[135] C. Nonaka, A. Oliveira, and C. Paiva, "Occurrence of antimicrobial residues in Brazilian food animals in 2008 and 2009," *Food Additives & Contaminants: Part A*, vol. 29, no. 4, pp. 526–534, 2012.

[136] L. Abramsson-Zetterberg, P. O. Darnerud, and S. Wretling, "Low intake of polycyclic aromatic hydrocarbons in Sweden: Results based on market basket data and a barbecue study," *Food and Chemical Toxicology*, vol. 74, pp. 107–111, 2014.

[137] C. Battisti, A. M. Girelli, and A. M. Tarola, "Polycyclic aromatic hydrocarbons (PAHs) in yogurt samples," *Food Additives & Contaminants: Part B. Surveillance*, vol. 8, no. 1, pp. 50–55, 2015.

[138] M. Ciecierska and M. W. Obiedziński, "Polycyclic aromatic hydrocarbons in the bakery chain," *Food Chemistry*, vol. 141, no. 1, pp. 1–9, 2013.

[139] H. Nakata, K. Uehara, Y. Goto et al., "Polycyclic aromatic hydrocarbons in oysters and sediments from the Yatsushiro Sea, Japan: Comparison of potential risks among PAHs, dioxins and dioxin-like compounds in benthic organisms," *Ecotoxicology and Environmental Safety*, vol. 99, pp. 61–68, 2014.

[140] H. P. V. Huynh and D. Nugegoda, "Effects of chlorpyrifos exposure on growth and food utilization in Australian catfish, Tandanus tandanus," *Bulletin of Environmental Contamination and Toxicology*, vol. 88, no. 1, pp. 25–29, 2012.

[141] M. Lu, W. W. Jiang, J. Wang et al., "Persistence and Dissipation of Chlorpyrifos in Brassica Chinensis, Lettuce, Celery, Asparagus Lettuce, Eggplant, and Pepper in a Greenhouse," *PLoS ONE*, vol. 9, no. 6, p. e100556, 2014.

[142] C. Benzidane and S. Dahamna, "Chlorpyrifos residues in food plant in the region of Setif-Algeria.," *Communications in Agricultural and Applied Biological Sciences*, vol. 78, no. 2, pp. 157–160, 2013.

[143] A. F. A. Mahmoud, Y. Ikenaka, Y. B. Yohannes et al., "Distribution and health risk assessment of organochlorine pesticides (OCPs) residue in edible cattle tissues from northeastern part of Egypt: High accumulation level of OCPs in tongue," *Chemosphere*, vol. 144, pp. 1365–1371, 2016.

[144] A. A. Raslan, S. Elbadry, and W. S. Darwish, "Estimation and Human Health Risk Assessment of Organochlorine Pesticides in Raw Milk Marketed in Zagazig City, Egypt," *Journal of Toxicology*, vol. 2018, pp. 1–8, 2018.

[145] L. A. Thompson, Y. Ikenaka, Y. B. Yohannes et al., "Concentrations and human health risk assessment of DDT and its metabolites in free-range and commercial chicken products from KwaZulu-Natal, South Africa," *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, vol. 34, no. 11, pp. 1959–1969, 2017.

[146] H. Deti, A. Hymete, A. A. Bekhit, A. M. I. Mohamed, and A. E.-D. A. Bekhit, "Persistent organochlorine pesticides residues in cow and goat milks collected from different regions of Ethiopia," *Chemosphere*, vol. 106, pp. 70–74, 2014.

[147] Y. Jeong, S. Lee, S. Kim et al., "Occurrence and exposure assessment of polychlorinated biphenyls and organochlorine pesticides from homemade baby food in Korea," *Science of the Total Environment*, vol. 470-471, pp. 1370–1375, 2014.

[148] F. M. Malhat, M. N. Haggag, N. M. Loutfy, M. A. M. Osman, and M. T. Ahmed, "Residues of organochlorine and synthetic pyrethroid pesticides in honey, an indicator of ambient environment, a pilot study," *Chemosphere*, vol. 120, pp. 457–461, 2015.

[149] M. Roszko, R. Jedrzejczak, and K. Szymczyk, "Polychlorinated biphenyls (PCBs), polychlorinated diphenyl ethers (PBDEs) and organochlorine pesticides in selected cereals available on the Polish retail market," *Science of the Total Environment*, vol. 466-467, pp. 136–151, 2014.

[150] J. Pan, N. Gai, H. Tang et al., "Organochlorine pesticides and polychlorinated biphenyls in grass, yak muscle, liver, and milk in Ruoergai high altitude prairie, the eastern edge of Qinghai-Tibet Plateau," *Science of the Total Environment*, vol. 491-492, pp. 131–137, 2014.

[151] Y. Ruiz, P. Suárez, A. Alonso, E. Longo, and F. San Juan, "Mutagenicity test using Vibrio harveyi in the assesment of water quality from mussel farms," *Water Research*, vol. 47, no. 8, pp. 2742–2756, 2013.

[152] Q.-H. Zhang, L. Huang, Y. Zhang, C.-H. Ke, and H.-Q. Huang, "Proteomic approach for identifying gonad differential proteins in the oyster (Crassostrea angulata) following food-chain contamination with HgCl2," *Journal of Proteomics*, vol. 94, pp. 37–53, 2013.

[153] M. Milan, S. Ferraresso, C. Ciofi et al., "Exploring the effects of seasonality and chemical pollution on the hepatopancreas transcriptome of the Manila clam," *Molecular Ecology*, vol. 22, no. 8, pp. 2157–2172, 2013.

[154] P. Koedrith, T. Thasiphu, J.-I. Weon, R. Boonprasert, K. Tuitemwong, and P. Tuitemwong, "Recent trends in rapid environmental monitoring of pathogens and toxicants: Potential of nanoparticle-based biosensor and applications," *The Scientific World Journal*, vol. 2015, 2015.

[155] J. C. Caldwell, M. V. Evans, and K. Krishnan, "Cutting edge PBPK models and analyses: Providing the basis for future modeling efforts and bridges to emerging toxicology paradigms," *Journal of Toxicology*, vol. 2012, 2012.

[156] "The European Commission, "REACH"," http://ec.europa.eu/environment/chemicals/reach/reach_en.htm.

[157] US Environmental Protection Agency, *Pesticide Registration Manual Helps Applicants*, https://www.epa.gov/pesticide-registration.

[158] J. Gago, L. Viñas, V. Besada, and J. Bellas, "The link between descriptors 8 and 9 of the Marine Strategy Framework Directive: lessons learnt in Spain," *Environmental Science and Pollution Research*, vol. 21, no. 23, pp. 13664–13671, 2014.

[159] M. Kolossa-Gehring, K. Becker, A. Conrad, C. Schröter-Kermani, C. Schulz, and M. Seiwert, "Environmental surveys, specimen bank and health related environmental monitoring in Germany," *International Journal of Hygiene and Environmental Health*, vol. 215, no. 2, pp. 120–126, 2012.

[160] P. Zhou, Y. Zhao, J. Li et al., "Dietary exposure to persistent organochlorine pesticides in 2007 Chinese total diet study," *Environment International*, vol. 42, no. 1, pp. 152–159, 2012.

[161] H.-Y. Lai, Z.-Y. Hseu, T.-C. Chen, B.-C. Chen, H.-Y. Guo, and Z.-S. Chen, "Health risk-based assessment and management of heavy metals-contaminated soil sites in Taiwan," *International Journal of Environmental Research and Public Health*, vol. 7, no. 10, pp. 3595–3614, 2010.

[162] Y. Liu, G. T. Kong, Q. Y. Jia et al., "Effects of soil properties on heavy metal accumulation in flowering chinese cabbage (brassica campestris L. ssp. chinensis var. utilis tsen et lee) in pearl river delta, China," *Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes*, vol. 42, no. 2, pp. 219–227, 2007.

[163] Y.-G. Zhu, "Soil science in the understanding of the security of food systems for health," *Asia Pacific Journal of Clinical Nutrition*, vol. 18, no. 4, pp. 516–519, 2009.

[164] H. L. Boul, "DDT residues in the environment—A review with a New Zealand perspective," *New Zealand Journal of Agricultural Research*, vol. 38, no. 2, pp. 257–277, 1995.

[165] G. K. Mishra, "Microbes in heavy metal remediation: A review on current trends and patents," *Recent Patents on Biotechnology*, vol. 11, no. 3, pp. 188–196, 2017.

[166] Q. Wu, J. Y. S. Leung, X. Huang et al., "Evaluation of the ability of black nightshade Solanum nigrum L. for phytoremediation of thallium-contaminated soil," *Environmental Science and Pollution Research*, vol. 22, no. 15, pp. 11478–11487, 2015.

[167] L. Yu, J. Zhu, Q. Huang, D. Su, R. Jiang, and H. Li, "Application of a rotation system to oilseed rape and rice fields in Cd-contaminated agricultural land to ensure food safety," *Ecotoxicology and Environmental Safety*, vol. 108, pp. 287–293, 2014.

[168] J.-J. Guo, X. Tan, H.-L. Fu et al., "Selection for Cd Pollution-Safe Cultivars of Chinese Kale (Brassica alboglabra L. H. Bailey) and Biochemical Mechanisms of the Cultivar-Dependent Cd Accumulation Involving in Cd Subcellular Distribution," *Journal of Agricultural and Food Chemistry*, vol. 66, no. 8, pp. 1923–1934, 2018.

[169] R.-R. Zhang, Y. Liu, W.-L. Xue, R.-X. Chen, S.-T. Du, and C.-W. Jin, "Slow-release nitrogen fertilizers can improve yield and

reduce Cd concentration in pakchoi (Brassica chinensis L.) grown in Cd-contaminated soil," *Environmental Science and Pollution Research*, vol. 23, no. 24, pp. 25074–25083, 2016.

[170] G. Liao, Q. Wu, R. Feng et al., "Efficiency evaluation for remediating paddy soil contaminated with cadmium and arsenic using water management, variety screening and foliage dressing technologies," *Journal of Environmental Management*, vol. 170, pp. 116–122, 2016.

[171] A. Rico, Y. Geng, A. Focks, and P. J. Van den Brink, "Modeling environmental and human health risks of veterinary medicinal products applied in pond aquaculture," *Environmental Toxicology and Chemistry*, vol. 32, no. 5, pp. 1196–1207, 2013.

[172] N. Maftoonazad and F. Badii, "Use of edible films and coatings to extend the shelf life of food products," *Recent Patents on Food, Nutrition & Agriculture*, vol. 1, no. 2, pp. 162–170, 2009.

[173] D. R. Roshan and R. J. Isaifan, "Household Air Pollution from Burning Biomass for Cooking and Heating – A Review of Health Hazards and Intervention Programs," *Journal of Environmental Science and Pollution Research*, vol. 4, no. 3, pp. 289–296, 2018.

Hindawi
Journal of Toxicology
Volume 2018, Article ID 7170510, 6 pages
https://doi.org/10.1155/2018/7170510

Research Article

Biosorption of Cadmium by Filamentous Fungi Isolated from Coastal Water and Sediments

Lebeth C. Manguilimotan and Jayzon G. Bitacura

Department of Biological Sciences, Visayas State University, Visca, Baybay City, Leyte 6521-A, Philippines

Correspondence should be addressed to Jayzon G. Bitacura; jayzon.bitacura@vsu.edu.ph

Received 24 May 2018; Revised 28 August 2018; Accepted 27 September 2018; Published 22 October 2018

Guest Editor: Wageh Sobhy Darwish

The use of microorganisms in decontaminating the environment encumbered with heavy metal pollutants through biosorption is considered as a good option for bioremediation. This study was conducted to isolate Cadmium (Cd) tolerant fungi from coastal waters and sediments, compare their biosorption capabilities, and identify the isolates with the highest Cd uptake. Water and sediment samples were collected near the effluent sites of industrial belt in Ibo, Lapu-lapu City, Cebu, Philippines. Potato dextrose agar (PDA) plates containing Cd (25, 50, 75, and 100 ppm) were used to isolate Cd tolerant fungi from the samples. The distinct colonies that grew on the highest Cd concentration (100 ppm) were then isolated into pure cultures. The pure cultures of Cd tolerant fungi served as a source of inocula for *in vitro* biosorption assay using Cd dissolved in potato dextrose broth (PDB) as the substrate. Cd tolerant fungal isolates with the highest Cd uptake were finally identified up to the lowest possible taxon based on their colonial and microscopic characteristics. Most filamentous fungal colonies have grown most at the lower Cd concentrations and least at the higher concentrations. From the characteristics of the fungal growth on the plate with the highest Cd concentration, eight distinct colonies from both sediment and water samples were isolated into pure cultures. Among the eight fungal isolates, only three had significant Cd biosorption efficiency, these were fungal isolate 3 (13.87 %), fungal isolate 6 (11.46 %), and fungal isolate 4 (10.71 %). Two of them (fungal isolates 3 and 4) belong to genus *Aspergillus* while the other (fungal isolate 6) is a species of *Penicillium*. The results of this study showed that Cd tolerant fungi with biosorption capacity could be isolated from coastal water and sediments in the vicinity of areas suspected of heavy metal contamination.

1. Introduction

Heavy metals are one of the constituting pollutants in water which are on the forefront of academic and regulatory concerns today. Metal effluents from the metal processing industries that are discharged into water bodies are not biodegraded but undergo chemical or microbial transformations, creating a large impact on the environment and public health [1, 2]. Recently, they have been found to be negatively affecting the gamete viability, fertilization, and embryonic development of *Tripneustes gratilla*, a marine invertebrate model organism [3]. Awareness of the importance of treatments and removal of heavy metals from such effluents to permissible limits before discharging into natural streams, rivers, and seas is rapidly growing worldwide.

Towards this direction, several conventional wastewater technologies were developed and are in use successfully at a large scale to reduce hazardous compounds concentration in wastewaters [4]. However, application of such traditional treatments is not economical. It requires continuous inputs of chemicals and it causes further environmental damages making it impractical. Hence, easy, effective, economic, and eco-friendly techniques are required for fine-tuning of wastewater management.

One of the identified reducing agents for heavy metals is the use of microorganisms like fungi [5–8]. Fungi can tolerate and detoxify metals in many ways. It could be through valence transformation, active uptake, precipitation inside or outside their cells, and biosorption [9–17]. Biosorption is a process of metal uptake by living or dead biomass through the binding of metal ion on the cell wall and extracellular materials [7].

The high surface-volume ratio of microorganisms and their ability to detoxify metals are among the reasons that they are considered as a potential alternative to synthetic resins for remediation of dilute solutions of metals and solid wastes [18]. The use of fungi, for instance, gained importance because it is eco-friendly, economical, and effective [19]. The cell wall of fungi consists of polysaccharides and proteins that offer multiple active sites for binding of metals [20]. The polysaccharides found in the cell walls of fungi are chitin and chitosan, which have been shown to sequester metal ions. The first step in biosorption is passive biosorption that occurs independently of cellular metabolism and proceeds rapidly by metal binding mechanisms like ion exchange, physical adsorption, coordination, complexation, or inorganic micro-precipitation. Passive biosorption is a reversible adsorption-desorption process. Elution could be done by other ions, chelating agents, or acids. Conversely, active biosorption occurs when metal ions penetrate the cell membrane and enter into the cells [20, 21].

Considering the mechanisms of metal resistance by fungi, it is expected that screening of metal tolerant fungi may provide strains with improved metal accumulation. Only limited studies have been conducted in the Philippines to systematically screen filamentous fungi from metal-polluted sites for their metal tolerance. Therefore, the isolation, characterization, and identification of Cadmium (Cd) tolerant indigenous fungi with biosorption potential from the coastal waters and sediments near the industrial plants in Barangay Ibo, Lapu-Lapu City, Cebu, Philippines, was made. Cd is one of the known environmental pollutants that are frequently encountered together in sewage and industrial wastewaters [22]. Hence the metal was chosen for this study.

2. Materials and Methods

2.1. Collection of Samples. Composite sediment and coastal water samples were collected 10 meters away from the effluent sites of the industrial plants in Barangay Ibo, Lapu-Lapu City, Cebu. A hand corer was used to obtain the sediment samples. Samples were obtained by pushing the corer up to 10 cm depth in the sediments. A water sampler was also used to collect coastal water samples. The water sampler was towed horizontally 5 meters away from the shore. Five replicates of water and sediment samples were collected at every sampling point. Three sampling points were considered in the site. These points are at least 100 meters apart.

The collected sample replicates from each sampling point were mixed thoroughly for uniform distribution of the fungal cells. From these composite samples, approximately 500 grams of sediments was placed in a sterile 500 mL glass beaker and was covered with aluminum foil [23] while approximately 1.25 L of water sample was placed in sterile ketchup bottles. Sediments and water samples were placed in an ice bucket and were transported immediately to the laboratory for microbiological analyses that were carried out within 48 hours after sample collection.

2.2. Isolation of Cadmium Tolerant Fungi. All the glassware used in the experiment was acid washed to avoid unwanted metal contamination. In a 250 mL beaker 10 g of composite sediment sample was suspended in 90 ml of sterilized distilled water and was shaken for 30 min. One mL (1 mL) of the diluted soil suspension and the water sample was spread plated on the previously prepared sterile potato dextrose agar (PDA) plates containing increasing cadmium (Cd) concentrations of 25 ppm, 50 ppm, 75 ppm, and 100 ppm. These concentrations fall in the range of the minimum inhibitory concentrations of some filamentous fungi on certain heavy metals [8]. On the other hand, plates with no Cd served as the negative control. The inoculated plates were incubated at 28°C for seven days. The morphologically distinct colonies that grew on the highest Cd concentration were considered as Cd tolerant fungi. According to the review of Igwe and Abia [24], biosorbents are inefficient when heavy metal concentrations reach 100 mg/L or 100 ppm. Thus, fungal colonies growing on these Cd concentrations could be more tolerant to Cd than those that grew at lower Cd levels. The colonies were purified on plates and slants of potato dextrose agar (PDA). These pure cultures served as a source of inoculums for the biosorption assay and for further characterization and identification.

2.3. Biosorption Assay. Prior to the assay, fungal isolates were grown for a week in a potato dextrose broth (PDB). Each of the isolates was assayed by adding 20 mL of the previously grown fungal biomass in sterile 250 mL Erlenmeyer flasks containing 100 mL of the previously prepared sterile PDB with 10 mL $CdSO_4$. Three replicates for every treatment were prepared. Three flasks containing $CdSO_4$ were not inoculated with fungal biomass serving as the negative controls. The initial concentration of Cd in the experimental and control flasks was first determined then the flasks were incubated at room temperature with constant shaking for five days [8]. After the incubation, the media from the inoculated flasks were centrifuged to separate the fungal mycelia from the broth. The supernatants produced after centrifugations were then analyzed for dissolved Cd.

The initial and final Cd concentrations were determined through Atomic Absorption Spectrophotometry (AAS) at the Central Analytical Services Laboratory of the PhilRootcrops Research and Training Center, Visayas State University. From the data gathered, the biosorption efficiencies of the fungal isolates were then evaluated using the equation below [25].

$$E = \left[\frac{(Ci - Cf)}{Ci}\right] x\, 100 \quad (1)$$

where

E is biosorption efficiency (%)

Ci is initial concentration of the metal in the solution

Cf is final concentration of the metal in the solution

2.4. Characterization and Identification of Cd Tolerant Fungal Isolates. The fungal isolates with the highest percentage of metal uptake were characterized and identified based on their colony shape, color, and spore formation as well as the

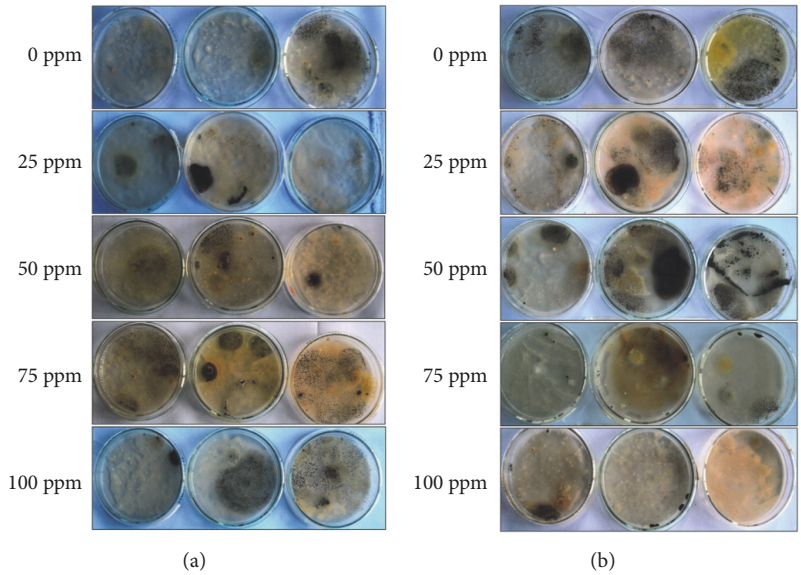


Figure 1: Seven-day-old fungal colonies from (a) water samples and (b) sediment samples isolated on PDA plates with 0 ppm, 25 ppm, 50 ppm, 75 ppm, and 100 ppm Cd concentrations.

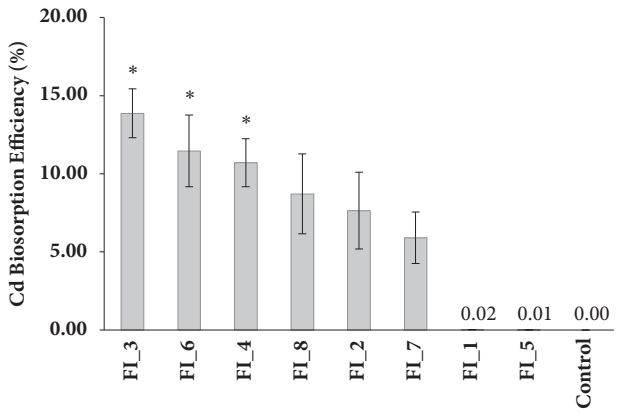


Figure 2: Cd biosorption efficiency (mean %±SE; n=3) of the fungal isolates after five days of biosorption assay; *($p \leq 0.05$) Tukey's HSD; FI=fungal isolate.

texture of fungal growth. Additionally, the microscopic characteristics of the isolates, like their conidia, conidiophores, and hyphae, were observed under an electric compound microscope (True Vision Microscope USA) at high power objective (400X) and oil immersion objective (1000X). Canon PowerShot A2200 digital camera was used to document the cell and colony morphology of the Cd tolerant fungal isolates.

2.5. Experimental Design and Statistical Analysis. The biosorption efficiency of the Cd tolerant fungal isolates was evaluated in Completely Randomized Design (CRD). Analysis of Variance (ANOVA) was used to determine the significant difference on the biosorption efficiencies of the Cd tolerant fungal isolates followed by post hoc multiple comparisons of means using Tukey's Honestly Significant Difference (HSD) Test to determine the isolates that show significant biosorption efficiencies.

3. Results and Discussion

Fungal colonies have grown on all plates containing different Cd concentrations. Most colonies have grown on lower Cd concentrations and least at higher concentrations (Figure 1). Five distinct colonies of fungi were isolated from the sediment samples and four distinct colonies of fungi from the water samples. However, after the pure culture, one isolate is found to have been isolated from both water and sediment samples, thus, giving a total of eight distinct fungal isolates.

Upon subjecting the eight fungal isolates to biosorption assay, it was found out that there was a significant difference in the biosorption efficiencies of the isolates ($p \leq 0.05$). Post hoc comparison of mean Cd biosorption efficiency revealed that fungal isolate 3 (13.87 %), fungal isolate 6 (11.46 %), and fungal isolate 4 (10.71 %) have significant biosorption capacities among all the fungal isolates. Following closely were fungal isolate 8 (8.71 %), fungal isolate 2 (7.64 %), and fungal isolate

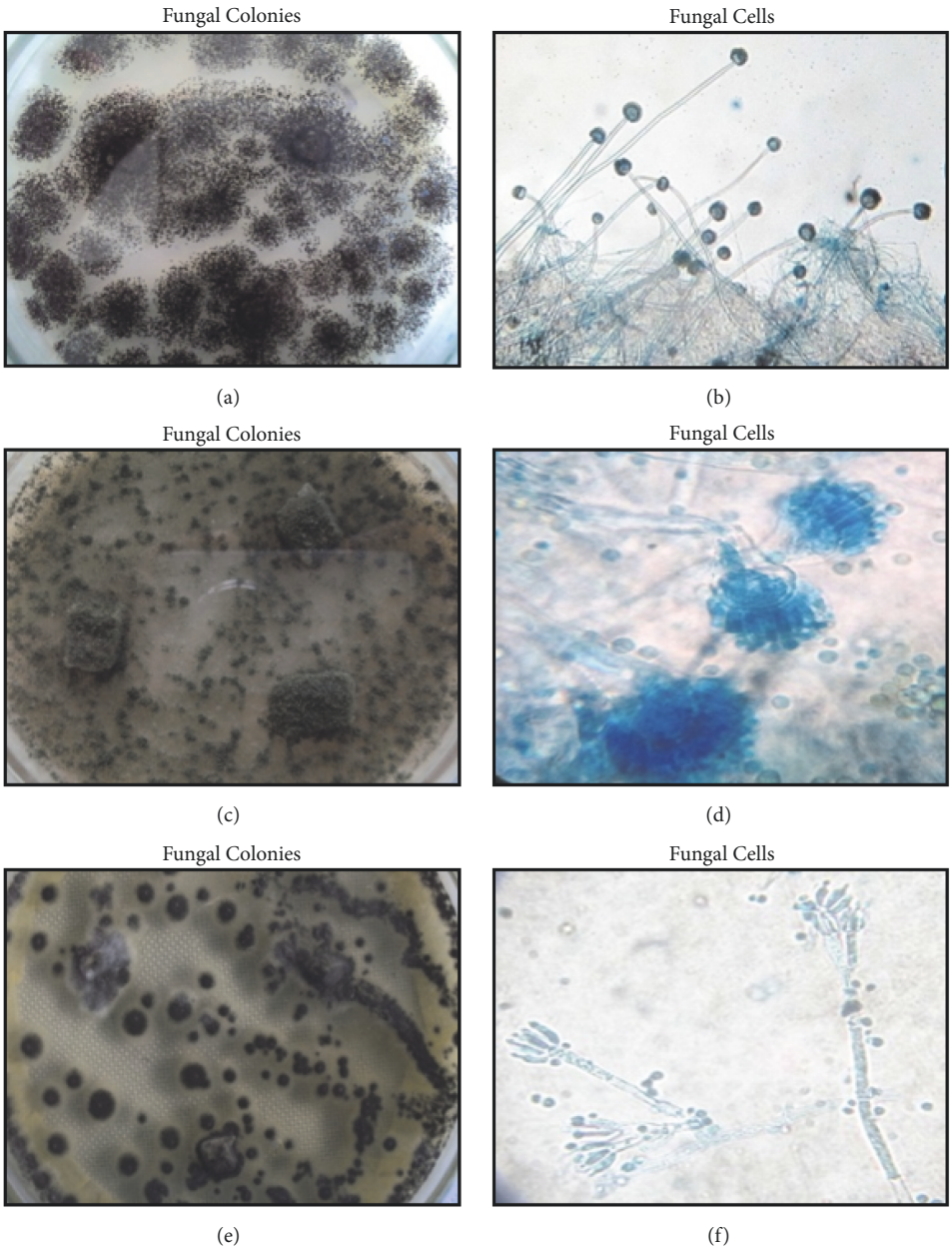


Figure 3: Colonial (left) and microscopic (right) characteristics (400X) of the three Cd tolerant fungal isolates with the highest percentage of Cd biosorption efficiency. Fungal isolates 3 (a and b) and 4 (c and d) were identified as *Aspergillus* spp. (a-d) while fungal isolate 6 (e and f) was identified as *Penicillium* sp.

7 (5.90 %), while fungal isolates 1 and 5 showed the least biosorption efficiencies at 0.02 % and 0.01 %, respectively (Figure 2).

Figure 3 shows the colonial and microscopic characteristics of the fungal isolates with the highest Cd uptake. Fungal isolate 3 consists of a compact dense layer of dark-brown to black colonies which are patching (Figure 3(a)). Conidial heads were short columnar and biseriate with the phialides borne on brown, septate metulae. Their conidiophores were turning dark towards the vesicle (Figure 3(b)). These are the characteristics of fungi belonging to genus *Aspergillus*. Colonies of fungal isolate 4 have a powdery texture. They were brownish green in color and became brownish-black as they aged. Their growth produced pale green exudates in the agar (Figure 3(c)). Microscopically, their conidial heads were radiating and columnar which form a brush-like structure (Figure 3(d)). This means that fungal isolate 4 belongs to the genus *Aspergillus* too. Finally, fungal isolate 6 colonies were fast growing; they were in shades of dark green and consist of dense felt conidiophores (Figure 3(e)). Microscopically, they have conidiophores that are hyaline and have a three-stage branch. They also have simple single-celled conidia (ameroconidia) in chains that were produced and attached in the basipetal succession of phialides, specialized

conidiogenous cells giving the brush-like appearance of the species (Figure 3(f)). These characteristics point to the genus *Penicillium*.

The occurrence of various filamentous fungi in sediments with heavy metals has also been reported in other works from different parts of the world [26, 27]. It may be because sediments contain nutrients and provide substrate needed for the growth of the vegetative hyphae of these filamentous fungal species. The sediments may also provide a place for microorganisms to hide against environmental changes, making them more stable. On the other hand, less fungal species were isolated in the water samples may be attributed to instability of water.

The variations in the Cd uptake among the different fungal biomass of different species may be related to their chemical characteristics [20]. Biosorption of this metal is based on ions associating with the cell surface wherein ion exchange and complexation reaction with functional groups like carboxyl, amides, hydroxyl, phosphate, and sulphydryl groups occur [8]. Kapoor and Viraraghavan [28] reported that carboxylate and amine groups are important in metal ion biosorption on by some filamentous fungal species.

Biosorption of heavy metals by fungi isolated from wastewater-contaminated sites have been reported by other researchers too. Faryal et al. [29] isolated filamentous fungi from the soil of the local textile industry. They were able to isolate *Aspergillus* sp., *Rhizopus* sp., *Rhodotorula* sp., *Drechslera* sp., and *Curvularia* sp. Parameswari et al. [8] isolated *Aspergillus niger*, *Phanerochaete chrysosporium*, and *Trichoderma viride* from municipal sewage contaminated with heavy metals. Among their isolates, *P. chrysosporium* accumulated 64.25 % of Cr and 57 % of Ni. Javaid and Bajwa [30] reported that *Pleurotus ostreatus* has 55% of biosorption efficiency for Cr (III) ions while Javaid et al. [25] revealed that *Schizophyllum commune* has biosorption efficiency of 72.01, 53.16, 7.08, and 19.87% for Cu (II), Ni (II), Zn (II), and Cr (VI) ions, respectively. These studies thus imply that biosorption efficiency of a certain fungal species may depend on the type of heavy metals they are subjected to. For instance, Khattab [31] reported that *Penicillium viridicatum* had high extraction activity for Pb but it had low dissolution activity for Zn and Cu from effluents.

Finally, biosorption of toxic metals depends on the extent of metabolic dependence [32]. The physiological state of the organism, the age of the cells, the availability of micronutrients during their growth, and the environmental conditions during the biosorption process (such as pH, temperature, and the presence of certain co-ions) are important parameters that affect the performance of a living biosorbent [33].

4. Conclusion

This study has shown that Cd tolerant fungi with biosorption capacity can be isolated from areas suspected of Cd contamination. Although further studies need to be done in order for these isolates to be used in bioremediation initiatives, this study opens a new venue of low cost and eco-friendly management of heavy metal pollutants.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors would like to thank Professor Yolanda C. Mangaoang of the Department of Pest Management for verifying the identification of fungal isolates and the Department of Biological Sciences for the use of chemicals and equipment needed in the experiments.

References

[1] A. Jakimska, P. Konieczka, K. Skóra, and J. Namieśnik, "Bioaccumulation of metals in tissues of marine animals, part I: The role and impact of heavy metals on organisms," *Polish Journal of Environmental Studies*, vol. 20, no. 5, pp. 1117–1125, 2011.

[2] V. Takarina and A. Adiwibowo, "Impact of heavy metals contamination on the biodiversity of marine benthic organisms in Jakarta Bay," *Journal of Coastal Development* , vol. 14, pp. 168–171, 2011.

[3] I. P. B. Tualla and J. G. Bitacura, "Effects of Cadmium and Zinc on the Gamete Viability, Fertilization, and Embryonic Development of Tripneustes gratilla (Linnaeus)," *Scientifica*, vol. 2016, 2016.

[4] N. Verma and R. Rehal, "Removal of Chronium by Albiziia libbeck pods from Industrial wastewater," *Journal of Indian Pollution Control*, vol. 12, no. 1, pp. 55–59, 1996.

[5] A. Kapoor and T. Viraraghavan, "Fungal biosorption - an alternative treatment option for heavy metal bearing wastewaters: a review," *Bioresource Technology*, vol. 53, no. 3, pp. 195–206, 1995.

[6] F. Veglio and F. Beolchini, "Removal of metals by biosorption: a review," *Hydrometallurgy*, vol. 44, no. 3, pp. 301–316, 1997.

[7] N. R. Bishnoi and Garima, "Fungus—an alternative for bioremediation of heavy metal containing wastewater: a review," *Journal of Scientific and Industrial Research*, vol. 64, no. 2, pp. 93–100, 2005.

[8] E. Parameswari, A. Lakshmanan, and T. Thilagavathi, "Biosorption and metal tolerance potential of filamentous fungi isolated from metal polluted ecosystem," *Electronic Journal of Environmental, Agricultural and Food Chemistry* , vol. 9, no. 4, pp. 664–671, 2010.

[9] R. S. Bai and T. E. Abraham, "Studies on chromium(VI) adsorption-desorption using immobilized fungal biomass," *Bioresource Technology*, vol. 87, no. 1, pp. 17–26, 2003.

[10] R. S. Prakasham, J. S. Merrie, R. Sheela, N. Saswathi, and S. V. Ramakrishna, "Biosorption of chromium VI by free and immobilized Rhizopus arrhizus," *Environmental Pollution*, vol. 104, no. 3, pp. 421–427, 1999.

[11] A. Kapoor, T. Viraraghavan, and D. R. Cullimore, "Removal of heavy metals using the fungus Aspergillus niger," *Bioresource Technology*, vol. 70, no. 1, pp. 95–104, 1999.

[12] G. Yan and T. Viraraghavan, "Heavy-metal removal from aqueous solution by fungus Mucor rouxii," *Water Research*, vol. 37, no. 18, pp. 4486–4496, 2003.

[13] Q. Li, S. Wu, G. Liu et al., "Simultaneous biosorption of cadmium (II) and lead (II) ions by pretreated biomass of Phanerochaete chrysosporium," *Separation and Purification Technology*, vol. 34, no. 1-3, pp. 135–142, 2004.

[14] L. Svecova, M. Spanelova, M. Kubal, and E. Guibal, "Cadmium, lead and mercury biosorption on waste fungal biomass issued from fermentation industry. I. Equilibrium studies," *Separation and Purification Technology*, vol. 52, no. 1, pp. 142–153, 2006.

[15] A. H. Hawari and C. N. Mulligan, "Biosorption of lead(II), cadmium(II), copper(II) and nickel(II) by anaerobic granular biomass," *Bioresource Technology*, vol. 97, no. 4, pp. 692–700, 2006.

[16] S. Čerňanský, M. Urík, J. Ševc, P. Littera, and E. Hiller, "Biosorption of arsenic and cadmium from aqueous solutions," *African Journal of Biotechnology*, vol. 6, no. 16, pp. 1932–1934, 2007.

[17] J.-M. Luo, X. Xiao, and S.-L. Luo, "Biosorption of cadmium(II) from aqueous solutions by industrial fungus Rhizopus cohnii," *Transactions of Nonferrous Metals Society of China*, vol. 20, no. 6, pp. 1104–1111, 2010.

[18] A. Magyarosy, R. Laidlaw, R. Kilaas, C. Echer, D. Clark, and J. Keasling, "Nickel accumulation and nickel oxalate precipitation by Aspergillus niger," *Applied Microbiology and Biotechnology*, vol. 59, no. 2-3, pp. 382–388, 2002.

[19] Y. Sag and T. Kutsal, "Recent trends in the biosorption of heavy metals: A review," *Biotechnology and Bioprocess Engineering*, vol. 6, no. 6, pp. 376–385, 2001.

[20] J. L. Wang and C. Chen, "Biosorbents for heavy metals removal and their future," *Biotechnology Advances*, vol. 27, no. 2, pp. 195–226, 2009.

[21] N. Das, R. Vimala, and P. Karthika, "Biosorption of heavy metals - An overview," *Indian Journal of Biochemistry and Biophysics*, vol. 7, no. 2, pp. 159–169, 2008.

[22] M. A. Oliver, "Soil and human health, a review," *European Journal of Soil Science*, vol. 48, no. 4, pp. 573–592, 1997.

[23] J. G. Bitacura, A. C. Balala, and P. P. Abit, "Fungi from coastal sediments as potential agents in biodegrading used engine oil," *Annals of Tropical Research*, vol. 34, no. 2, pp. 112–125, 2012.

[24] J. C. Igwe and A. A. Abia, "A bioseparation process for removing heavy metals from waste water using biosorbents," *African Journal of Biotechnology*, vol. 5, no. 12, pp. 1167–1179, 2006.

[25] J. Amna, R. Bajwa, and A. Javaid, "Biosorption of heavy metals using a dead macro fungus Schizophyllum Commune fries: Evaluation of equilibrium and kinetic models," *Pakistan Journal of Botany*, vol. 42, no. 3, pp. 2105–2118, 2010.

[26] L. A. J. Shittu, M. P. Zachariah, G. Ajayi, J. A. Oguntola, M. C. Izegbu, and O. A. Ashiru, "The negative impacts of adolescent sexuality problems among secondary school students in Oworonshoki, Lagos," *Scientific Research and Essays*, vol. 2, no. 1, pp. 23–28, 2007.

[27] S. Zafar, F. Aqil, and I. Ahmad, "Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil," *Bioresource Technology*, vol. 98, no. 13, pp. 2557–2561, 2007.

[28] A. Kapoor and T. Viraraghavan, "Heavy metal biosorption sites in Aspergillus niger," *Bioresource Technology*, vol. 61, no. 3, pp. 221–227, 1997.

[29] R. Faryal, A. Lodhi, and A. Hameed, "Isolation, characterization and biosorption of zinc by indigenous fungal strains Aspergillus fumigatus RH05 and Aspergillus flavus RH07," *Pakistan Journal of Botany*, vol. 38, no. 3, pp. 817–832, 2006.

[30] A. Javaid and R. Bajwa, "Biosorption of Cr(III) ions from tannery wastewater by Pleurotus ostreatus," *Mycopathology*, vol. 5, no. 2, pp. 71–79, 2007.

[31] O. K. H. Khattab, "Bisorption of heavy metals using aquatic pycnidial and hyptomycetes fungi," *Journal of Biological Sciences*, vol. 7, no. 2, pp. 374–378, 2007.

[32] G. M. Gadd, "Interactions of fungi with toxic metals," *New Phytologist*, vol. 124, no. 1, pp. 25–60, 1993.

[33] R. Say, A. Denizli, and M. Yakup Arica, "Biosorption of cadmium(II), lead(II) and copper(II) with the filamentous fungus Phanerochaete chrysosporium," *Bioresource Technology*, vol. 76, no. 1, pp. 67–70, 2001.

Hindawi
Journal of Toxicology
Volume 2018, Article ID 3821797, 8 pages
https://doi.org/10.1155/2018/3821797

Research Article

Estimation and Human Health Risk Assessment of Organochlorine Pesticides in Raw Milk Marketed in Zagazig City, Egypt

Amal A. Raslan,[1] Seham Elbadry,[1] and Wageh Sobhy Darwish [2]

[1] *Educational Veterinary Hospital, Faculty of Veterinary Medicine, Zagazig University, 44519, Egypt*
[2] *Food Control Department, Faculty of Veterinary Medicine, Zagazig University, 44519, Egypt*

Correspondence should be addressed to Wageh Sobhy Darwish; wagehdarwish@zu.edu.eg

Received 21 May 2018; Revised 20 July 2018; Accepted 1 August 2018; Published 12 August 2018

Academic Editor: Valerio Matozzo

Milk is nearly a perfect natural food and is widely used by all segments of our population especially for infants and the elderly. Organochlorine pesticides (OCPs) have been used worldwide, particularly in many African countries as in Egypt for the control of pests. OCPs are characterized by their bioaccumulation in the environment, especially in the food chain, where they find their way into the human body. The objectives of this study were initially to estimate the residual concentrations of different OCPs in three kinds of fresh and raw milk from different animals (cattle, buffalo, and goat) marketed in Egypt. Additionally, human dietary intake and risk assessment of OCPs were calculated. The tested OCPs included pp-DDT and its metabolites pp-DDD and pp-DDE; hexachlorohexanes (HCHs) including α HCH and γ HCH; heptachlor and heptachlor epoxide; aldrin and endrin; chlordane, methoxychlor, and hexachloride benzene. The recorded results revealed that goat and buffalo milk samples had the highest incidence of OCPs' contamination (75% for each), while this percentage was 50% in cow's milk. The mean values of ΣOCPs were 317.83 ± 34.11, 605 ± 50.54, and 1210.57 ± 99.55 (ppb/ww) in the examined cattle, buffalo, and goat milk samples, respectively. All examined OCPs were within the maximum permissible limits (MPLs) set by World Health Organization with only 10% of goat milk samples exceeding this MRL. The estimated daily intake, noncancer, and cancer health risk assessment of the tested OCPs revealed the potential cancer risk especially among children consuming goat's milk. The public health importance of such OCPs was discussed.

1. Introduction

Milk is a complex, bioactive substance to promote growth and development of infant mammals. Cow, buffalo, and goat milk are widely consumed around the world, especially in Egypt. In fact, milk is considered as an ideal source of macroelements such as calcium, phosphorus, and potassium [1].

The widespread occurrence of any foreign chemical in the environment is a matter of public health concern. Pesticides are extensively used to increase agricultural products through preventing losses due to agricultural pests. The health authorities also use these chemicals to control various vectors, which spread diseases like malaria and plague [2].

Among the major groups of pesticides, organochlorines are more potent due to their persistence and stability. Universally important organochlorine pesticides (OCPs) are para, para, dichlorodiphenyltrichloroethane (pp-DDT), hexachloride benzene (HCB), chlordane, heptachlor, aldrin, dieldrin, and endrin. Due to the lipophilic nature of these pesticides, milk and other fat-rich substances are the key items for their accumulation [3]. These toxicants get into the human body through the food chain and cause serious health hazards [4].

Egypt as one of the most populous countries in Africa depend mainly on agricultural activities as major sources of national income. Therefore, pesticides are frequently used in Egypt to control pests or directly spread into animal skin for prevention and control of external parasites. These chemicals may find their way into animal body and subsequently pass into milk causing several toxicological implications for both animal and human if contaminated milk or other dairy products were consumed [5]. Studies had been done to investigate OCPs residues in different kinds of food including

milk and other dairy products worldwide. However, in Egypt, few reports had surveyed the residual levels of OCPs in milk. In addition, the dietary intake and human health risk assessment due to consumption of the contaminated milk in Egypt is less informed.

Due to the previous facts, this study was conducted to firstly investigate the residual concentrations of OCPs in the milk of cattle, buffalo, and goat in Egypt. The tested OCPs included pp-DDT and its metabolites pp-DDD and pp-DDE; hexachlorohexanes (HCHs) including α HCH and γ HCH; heptachlor and heptachlor epoxide; aldrin and endrin; chlordane, methoxychlor, and HCB. Secondly, the dietary intake, carcinogenic, and noncarcinogenic risks due to consumption of such contaminated milk were calculated.

2. Materials and Methods

All experiments were done according to the rules and guidelines of Zagazig University, Egypt.

2.1. Sampling. Sixty milk samples (20 each of cow, buffalo, and goat milk) were randomly purchased from markets in Zagazig city, Sharkia province, Egypt. Raw milk is sold in Egypt in polyethylene bags, and each sample weighs 500 g. Samples were transferred into laboratory in a cooled container. Organochlorine pesticides were extracted and measured at Agricultural Research Center, Dokki, Giza, Egypt.

2.2. Detection of Organochlorine Compounds

2.2.1. Chemicals. Standard OCPs including pp-DDT, pp-DDD, pp-DDE, α HCH, γ HCH, heptachlor, heptachlor epoxide, aldrin, endrin, chlordane, methoxychlor, and HCB were obtained from Sigma–Aldrich (Germany). Petroleum ether, diethyl ether, n-hexane, acetonitrile, anhydrous sodium sulfate, and methylene chloride were bought from Merck (Darmstadt, Germany). Florisil (PR Grade, 60–100 mesh) was purchased from Silica (Silica Co., USA). All solvents were of pesticide residue grade and subjected to a solvent purity test for residue analysis suitability. Florisil was activated at 130°C for 24 h and cooled to room temperature.

2.2.2. Extraction and Preparation of Samples. Each individual sample (50 ml) was mixed with anhydrous sodium sulfate (100 g) and petroleum ether (150, 100, and 100 ml, respectively) in three successive extraction steps for 2 min each, as described before [6]. Anhydrous sodium sulfate removes water and helps to disintegrate the sample. Samples were filtered with a vacuum pump after each extraction. The solvent was evaporated on a rotary evaporator at 40°C until dryness.

2.2.3. Partitioning of the Extract. Partitioning of the extracted samples was carried out according to the method of the Association of Official Analytical Chemists [7]. At first 500 ml n-hexane was partitioned with an equal volume of acetonitrile by mixing these two solvents in a separating funnel followed by separation of each solvent to be used for sample partitioning. The extracted sample was transferred with a mixture of 80 ml n-hexane and 20 ml acetonitrile into a 100-ml separating funnel, followed by vigorous shaking for 2 min. After separation of two solvent layers, acetonitrile was collected in a flask after being passed through anhydrous sodium sulfate to remove any moisture. Another 20 ml acetonitrile was added to n-hexane and the aforementioned partitioning step was repeated 3 times. Finally, n-hexane was discarded while acetonitrile was evaporated on a rotary evaporator to a volume less than 10 ml to be used for florisil cleanup.

2.2.4. Cleanup of the Extract. Cleanup of the extracted samples, to remove the residual fat, was performed by transferring the extract into a glass chromatographic column (22 mm i.d.) containing 20 g activated florisil (60–100 mesh) topped with 1-cm layer of anhydrous sodium sulfate. The prepared column was firstly rinsed with 50 ml petroleum ether, and then the extracted sample was transferred onto the column. The column was eluted with 200 ml eluent (10% anhydrous diethyl ether + 90% petroleum ether) followed by a second elution with 100 ml of another eluent (1% acetonitrile + 29% n-hexane + 70% methylene chloride). The collected eluent was concentrated on a rotary evaporator and dissolved in hexane to a volume of 10 ml. An aliquot of each extract was transferred to 2-ml injection vials to be ready for the analysis with the electron capture gas chromatography.

2.2.5. Determination of Organochlorine Pesticide Residual Concentrations. Organochlorine residues were determined by analysis of samples using electron capture gas chromatography (Hewlett Packard GC Model 6890) equipped with Ni63–electron capture detector. GC conditions were HP- 5MS capillary column (30m length X 0.32mm internal diameter (i.d..), X 0.25μm film thickness; carrier gas: N_2 at a flow rate of 4 ml/min; injector and detector temperatures were 230°C and 300°C, respectively). The extract was injected into a single inlet that was split into the dual columns. Instrumental settings were as follows: injector and detector temperatures were 230°C and 300°C, respectively; the gas chromatography oven temperature program was initiated at 150°C for 5 min, raised to 170°C (at a rate of 5°C/min) and held for 10 min, then raised to 220°C (at a rate of 10°C/min) and held for 20 min (with a total run time of 44 min); the injection volume was 1, ll, and the flow rates of nitrogen make-up gas were 20 ml/min.

2.2.6. Quality Assurance of Analytical Procedures. Calibration standard curves were created and the organochlorine pesticide residues were quantitatively determined by comparison with the standard solutions injected under the identical gas chromatography conditions. The standard reference material, SRM 1947 (Lake Michigan Fish Tissue), was analyzed during the analysis of samples followed by the same procedure of extraction, cleanup, and analysis. The percentage of recoveries of the organochlorines tested ranged from 86% to 109%. Residue levels for each pesticide were subsequently corrected for the recovery values. The limits of detection (LOD) and quantification (LOQ) for the tested OCPs were based on 3:1

signal to noise ratio (S/N) and ranged from 0.004 to 0.20 ng g^{-1} (LOD) and 0.024 to 0.036 ng g^{-1} (LOQ).

2.3. Human Health Risk Assessment. To estimate human health risks due to ingestion of OCPs contaminated milk among Egyptian populations (children and adults), both estimated daily intake (EDI) and hazard ratio (HR) were calculated based on the equations recommended by USEPA [8].

$$\text{EDI} = \text{C} * \frac{\text{Fi}}{\text{Bwt}} \quad (1)$$

where C is the average concentration of the chemical contaminants (ng g/ww) in the milk. Fi is the average daily intake, based on the information retrieved from Egyptian consumers; Fi was set to be 200 g and 400 g for adults and children, respectively. Bwt is the average body weight for Egyptian adults (60 kg) and children (30 kg) [9]. EDIs were compared with the acceptable daily intake (ADI) [10].

Noncancer and cancer risk assessment were calculated using hazard ratio (HR). A hazard ratio higher than one indicates potential human health risks [11].

$$\text{HRs} = \frac{\text{EDI}}{\text{BMC}} \quad (2)$$

The benchmark concentration (BMC) for carcinogenic effects was derived from cancer slope factor (CSF) and for noncarcinogenic effects was based on the oral reference dose (RFD). Both CSF and RFD were obtained from the United States Environmental Protection Agency Integrated Risk Information System [8].

2.4. Statistical Analysis. All values are expressed as means ± SE, and all measurements were carried out in duplicate. Statistical significance was evaluated using the comparative of means method (the Tukey–Kramer HSD test) (JMP statistical package; SAS Institute Inc., Cary, NC).

3. Results and Discussion

Organochlorine pesticides (OCPs) have been used worldwide, particularly in Africa for several decades. Although many are banned, several African countries still use OCPs especially for the prevention and control of malaria. OCPs are characterized by their bioaccumulation in the environment, especially in the food chain, where they find their way into the human body.

3.1. Residue Levels of OCPs in Marketed Milk in Egypt. In this study, the residual concentrations of different OCPs in three kinds of marketed milk (cattle, buffalo, and goat) in Egypt were estimated.

The recorded results revealed that goat and buffalo milk samples had the highest contamination level of OCPs (75%, 15 out of 20 examined samples), while this percentage was 50% (10 out of 20 examined samples) in cow's milk (see Figure 1). OCP-positive samples in the current investigation was lower than those detected by Heck *et al.* [12], who reported positive frequencies (100%) in the examined buffalo and sheep milk samples marketed in Brazil.

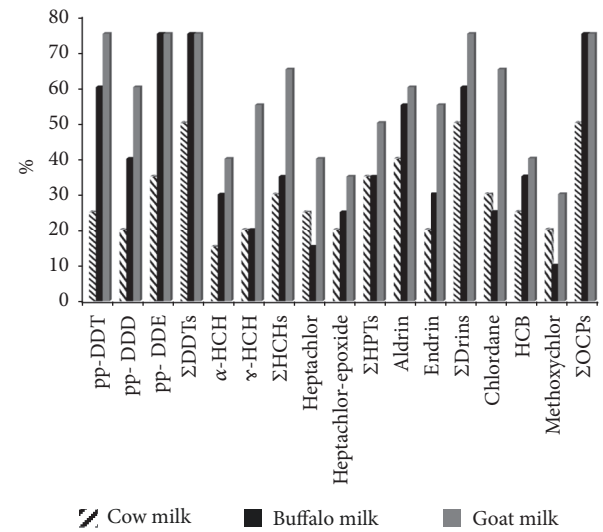


Figure 1: Frequency (%) of individual and total OCPs contamination of the examined milk samples from different animal species (n=20 each).

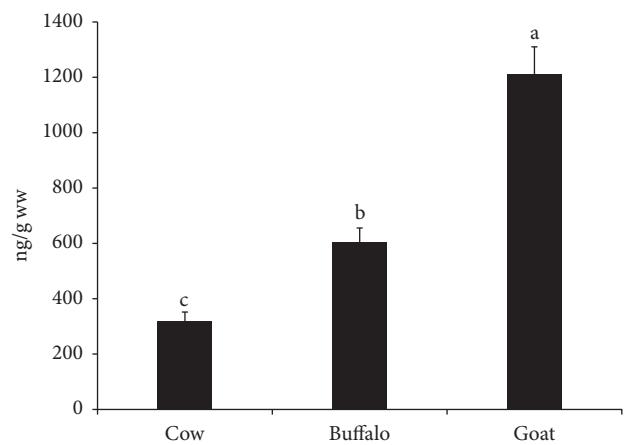


Figure 2: **Total organochlorine pesticide residues (OCPs) in the examined milk samples.** Data represent mean ± SE (ng/g ww) for total OCPs in the examined milk samples from different animal species (n=20 each). Columns that carry different superscript letter are significantly different at $p<0.05$.

The mean values of ΣOCPs in the examined milk samples were 317.83 ± 34.11, 605 ± 50.54, and 1210.57 ± 99.55 (ng/g ww) in the examined cattle, buffalo, and goat milk samples, respectively (see Figure 2). The recorded concentrations in this study were much lower than the concentrations recorded in buffalo milk by in India (8571 ng/g ww) [13]. However, these concentrations were comparable to 874.40 and 485.76 (ng/g ww) that reported in goat's milk and cheese retailed in Ethiopia and Ghana, respectively [14, 15].

Although OCPs' use has been banned in Egypt since the 1980s, DDTs are still detected in various foods in the country. For instance, mussels from Abu Qir Bay contained several OCPs, with DDT concentrations up to 31000 (ng/g dw), but a risk assessment showed no expected adverse

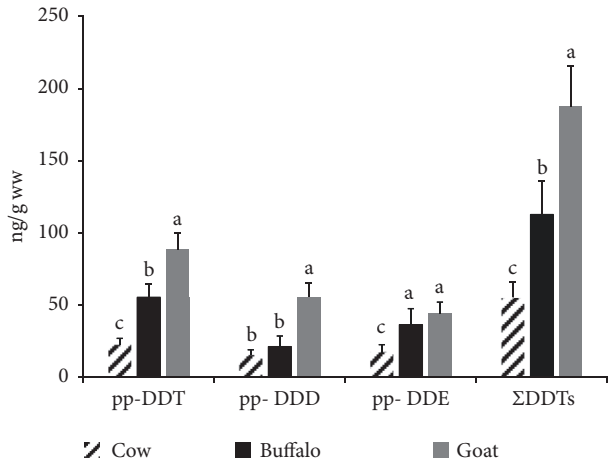


Figure 3: **Total DDT and its metabolites in the examined milk samples.** Data represent mean ± SE (ng/g ww) for total DDT and its metabolites in the examined milk samples from different animal species (n=20 each). Columns that carry different superscript letter among the same chemical are significantly different at $p<0.05$.

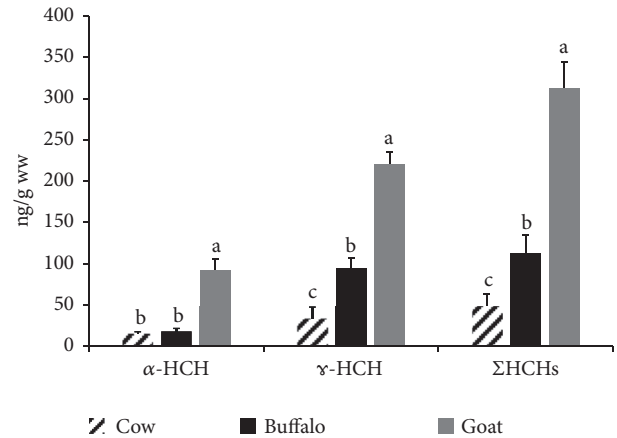


Figure 4: **Total HCH and its isomers' residues in the examined milk samples.** Data represent mean ± SE (ng/g ww) for total HCH and its isomers in the examined milk samples from different animal species (n=20 each). Columns that carry different superscript letter among the same chemical are significantly different at $p<0.05$.

effects on people through mussel consumption [16]. In the present study, either pp-DDT or its metabolites pp-DDD and pp-DDE were detected in 50%, 75%, and 75% of the examined cattle, buffalo, and goat milk samples, respectively (see Figure 1). The frequency of detection of OCPs in this study was comparable to that detected in raw milk from other countries (35-75%) as in China, India, Mexico, and Slovakia [13, 17–19]. The recorded residual concentrations of the detected DDTs were graphed (see Figure 3). Goat milk had significantly ($p< 0.05$) the highest ΣDDTs followed by buffalo and cattle milk samples. The average concentrations were 187.49 ± 27.88, 112.66 ± 23.11, and 54.77 ± 11.14 (ng/g ww) in the goat, buffalo, and cattle milk samples, respectively (see Figure 3). These results also show that the detected concentrations of either pp-DDT or its metabolites pp-DDE and pp-DDD were low, when compared with the established maximum residual concentrations (MRLs) (200 ng/g ww) by World Health Organization [20]. Presence of residues of DDTs in the milk samples indicate the past use of these pesticides in the agricultural activities in Egypt. In correspondence to the results of this study, Darko and Acquaah [14] detected DDTs in milk, yoghurt, and cheese marketed in Ghana in concentrations ranged from 0.01 to 119 ng/g ww. Unlikely, higher concentrations of DDTs (1230 and 874.4 ng/g ww) were recorded in cattle and goat milk samples collected from Ethiopian markets [15]. In contrast, Shaker and Elsharkawy [21] did not detect DDTs in the buffalo milk samples collected from Assuit city, Egypt.

Hexachlorocyclohexanes (HCHs) were detected in 30%, 35%, and 65% of the examined cattle, buffalo, and goat milk samples, respectively (see Figure 1). Data presented in Figure 4 represent ΣHCHs and its α-HCH and ɤ-HCH isomers. The average ΣHCHs values were 48.65 ± 15.12, 113.27 ± 21.23, and 313.16 ± 31.11 (ng/g ww) in the examined cattle, buffalo, and goat milk samples, respectively. Lindane (ɤ-HCH) is the most active and stable isomer of HCHs. The residual concentrations of lindane in the examined milk samples were 34.44 ± 14.1, 95.11 ± 11.33, and 221.00 ± 14.25 (ng/g ww) in cattle, buffalo, and goat milk samples, respectively. It is clear that goat had significantly the highest α-HCH, ɤ-HCH, and ΣHCHs followed by buffalo and finally cattle samples (see Figure 4). Codex Alimentarius Commission set MRLs of lindane to be 200 (ng/g ww) [22], with only 10% (2 out of 20) of goat milk samples exceeding this MRL. The recorded concentrations in this study go in agreement with the detected concentrations of ΣHCHs in buffalo liver, kidney, and tongue (34.97-351.57 ng/g lw) collected from Zagazig slaughter house [23].

Furthermore, the concentrations of HCHs in this study were comparable to the recorded concentrations in cattle raw milk marketed in Egypt, India, Ghana, Mexico, and Uganda [13, 14, 18, 21, 24].

Heptachlor and its epoxide were detected in 35%, 35%, and 50% of the examined cattle, buffalo, and goat milk samples, respectively (see Figure 1). The sum values of heptachlor and its metabolite were 31.88 ± 8.23, 38.63 ± 8.22, and 28.88 ± 3.56 (ng/g ww) in the examined cattle, buffalo, and goat milk samples with no significant differences among examined species (see Figure 5). The recorded concentrations of Σheptachlors in the current study were lower than that reported in buffalo milk in India (335 ng/g ww) [13]. None of the examined samples in the present study exceeded MPLs of heptachlors (150 ppb) [20].

Drins either aldrin or endrin were detected in 50%, 60%, and 75% of the examined cattle, buffalo, and goat milk samples, respectively (see Figure 1). The mean residual concentrations of the total drins were 13.96 ± 2.44, 25.95 ± 4.16, and 59.43 ± 8.44 (ng/g ww) in the examined cattle, buffalo, and goat milk samples with the goat milk in the top of the examined species (see Figure 6). All examined samples were within total drins' MPL (150 ppb) [20]. The concentrations of the Σdrins in buffalo's milk in the present study were comparable to the recorded values in fresh

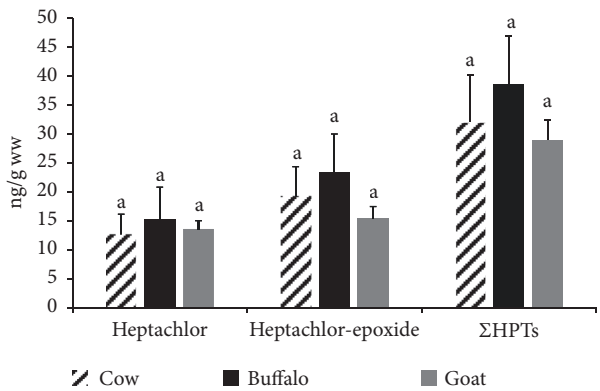


Figure 5: **Total heptachlor and its epoxide metabolite in the examined milk samples.** Data represent mean ± SE (ng/g ww) for total heptachlor and its epoxide in the examined milk samples from different animal species (n=20 each). Columns that carry same superscript letter among the same chemical are not significantly different at $p<0.05$.

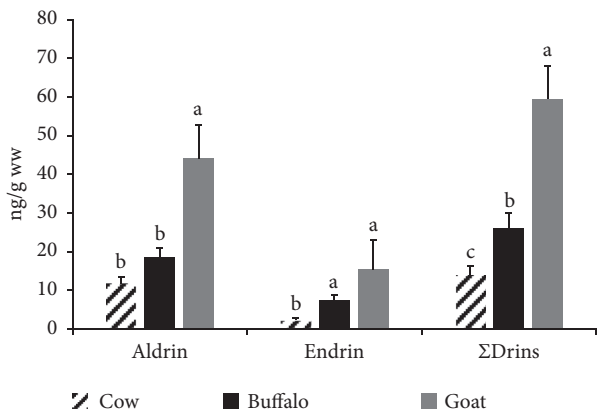


Figure 6: **Total drins in the examined milk samples.** Data represent mean ± SE (ng/g ww) for total drins in the examined milk samples from different animal species (n=20 each). Columns that carry same superscript letter among the same chemical are not significantly different at $p<0.05$.

buffalo's milk in Egypt (15 ng/g ww) and in cheese in Ghana (7.88 ng/g ww) [14, 21].

Other examined OCPs such as chlordane, HCB, and methoxychlor were detected in 10-60% (see Figure 1). The positive samples had minute concentrations of these OCPs that ranged from 1.55 to 14.21 (ng/g ww) (see Figure 7); all samples were below MPLs [20]. Nearly, similar values were reported in Assiut, Egypt [21].

It is worth noting that in the present work goat milk had the highest OCPs residues. This may be due to the grazing behavior of the goat. Additionally, buffalo's milk had higher OCPs compared with the milk of the cow. This may be attributed either to the high fat content (7.47%) of the buffalo's milk or to the dietary habits of the buffalo, like different fodder and to variations in diets compared with the cows [13].

3.2. Human Dietary Intake and Risk Assessment of OCPs. Humans can be exposed to OCPs via several routes including

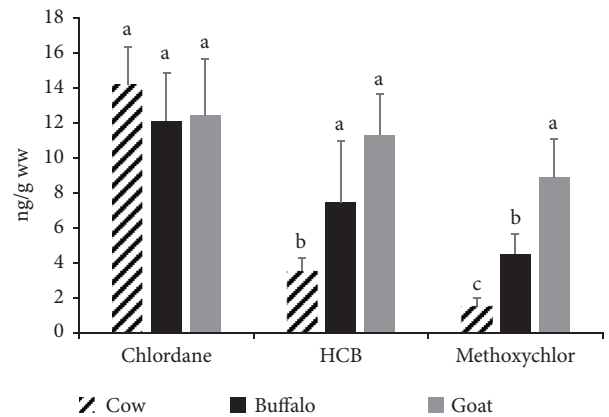


Figure 7: **Chlordane, HCB, and methoxychlor residues in the examined milk samples.** Data represent mean ± SE (ng/g ww) for chlordane, HCB, and methoxychlor residues in the examined milk samples from different animal species (n=20 each). Columns that carry same superscript letter among the same chemical are not significantly different at $p<0.05$.

breathing of polluted air, dermal penetration, or ingestion of contaminated foods and drinking water. OCP-contaminated foods like milk and other dairy products are considered the main source of human exposure to pesticides [25]. In the current study, EDIs of different OCPs were presented in Table 1. In general, the calculated EDIs were far below the acceptable daily intakes [10]. However, among the analyzed samples, consumption of goat's milk especially by children is alarming for heptachlors and drins. The low dietary intake of other OCPs may be due to the restriction of the use of OCPs in the agricultural activities. In correspondence with EDIs of OCPs in milk, Mahmoud *et al.* [26] reported relatively similar EDIs for OCPs via consumption of meat and offal marketed in Egypt.

The analyzed OCPs in the present study had both cancer and noncancer risks [27]. Cancer and noncancer hazard ratios through consumption of milk in Egypt for both adults and children were summarized in Tables 2 and 3. Noncancer HR values were far below one in all analyzed OCPs except for methoxychlor (see Table 2). However, the lifetime cancer risks were considered high in the present study, especially for DDTs and HCHs among children consuming goat's milk (see Table 3). Similarly, cancer HR greater than one were reported in studies conducted in Egypt and Mexico [26, 28].

Maternal transfer is also possible across the placenta to the foetus or via breast milk to infants. Residue levels of these compounds in living organisms depend on each organism's habitat and position in the food chain [29]. OCPs are classified among the endocrine disrupting chemicals [30], which are linked to several toxicological implications that include reduced fertility, spontaneous abortion [31], and reproductive tract anomalies among both sexes [32].

In conclusion, high concentration of the tested OCPs reveals the increased improper use of these pesticides by the farmers for agricultural purposes. These pollutants are characterized by long persistence in the environment and thus may pass to next generations of humans and different plant

Table 1: Estimated daily intake of OCPs due to ingestion of milk among Egyptian population.

	ADI	Cow's milk		Buffalo's milk		Goat's milk	
		Adults	Children	Adults	Children	Adults	Children
pp-DDT	10000	74.03	296.13	184.43	737.73	293.7	1174.8
pp-DDD	10000	51.1	204.4	70.36	281.46	184.1	736.4
pp-DDE	10000	57.43	229.73	120.73	482.93	147.16	588.66
Heptachlor	100	42.23	168.93	50.76	203.06	45.16	180.66
Heptachlor-epoxide	100	64.03	256.13	78	312	51.1	204.4
α-HCH	5000	50.7	202.8	60.53	242.13	307.2	1228.8
γ-HCH	5000	111.46	445.86	317.03	1268.13	736.66	2946.66
Aldrin	100	38.86	**155.46**	61.83	**247.33**	**147.03**	**588.13**
Endrin	100	7.66	30.66	24.66	98.66	51.06	**204.26**
Chlordane	500	47.36	189.46	40.33	161.33	41.46	165.86
HCB	600	11.83	47.33	24.8	99.2	37.76	151.06
Methoxychlor	6300	5.16	20.66	15	60	29.6	118.4

ADI: acceptable daily intake.
Values in bold are higher than ADI.

Table 2: Noncancer hazard ratio among Egyptian population due to ingestion of OCPs-contaminated milk from different animal species.

	RFD	Cow's milk		Buffalo's milk		Goat's milk	
		Adults	Children	Adults	Children	Adults	Children
pp-DDT	5.00E-04	0.04	0.15	0.09	0.37	0.15	0.59
pp-DDD	5.00E-04	0.03	0.1	0.04	0.14	0.09	0.37
pp-DDE	5.00E-04	0.03	0.11	0.06	0.24	0.07	0.29
Heptachlor	5.00E-04	0.02	0.08	0.03	0.1	0.02	0.09
Heptachlor-epoxide	5.00E-04	0.03	0.13	0.04	0.16	0.03	0.1
α-HCH	3.00E-04	0.02	0.06	0.02	0.07	0.09	0.37
γ-HCH	3.00E-04	0.03	0.13	0.09	0.38	0.22	0.88
Aldrin	3.00E-04	0.01	0.05	0.02	0.07	0.04	0.18
Endrin	3.00E-04	0.002	0.01	0.01	0.03	0.02	0.06
Chlordane	5.00E-04	0.02	0.09	0.02	0.08	0.02	0.08
HCB	8.00E-04	0.01	0.04	0.02	0.08	0.03	0.12
Methoxychlor	0.05	0.26	**1.03**	0.75	**3**	**1.48**	**5.92**

RFD: oral reference doses.
Values in bold represent higher hazard ratio (>1.0).

Table 3: Cancer hazard ratio among Egyptian population due to ingestion of OCPs-contaminated milk from different animal species.

	CSF	Cow milk		Buffalo milk		Goat milk	
		Adults	Children	Adults	Children	Adults	Children
pp-DDT	0.34	0.22	0.88	0.54	**2.17**	0.86	**3.46**
pp-DDD	0.24	0.21	0.85	0.29	**1.17**	0.77	**3.07**
pp-DDE	0.34	0.17	0.68	0.36	**1.42**	0.43	**1.73**
Heptachlor	4.5	0.01	0.04	0.01	0.05	0.01	0.05
Heptachlor-epoxide	4.5	0.01	0.06	0.02	0.07	0.01	0.05
α-HCH	1.1	0.05	0.18	0.06	0.22	0.28	**1.12**
γ-HCH	1.1	0.1	0.41	0.29	**1.15**	0.67	**2.68**
Aldrin	17	0.002	0.009	0.004	0.015	0.01	0.03
Endrin	17	0.0004	0.002	0.001	0.006	0.003	0.01
Chlordane	0.35	0.14	0.54	0.115	0.46	0.12	0.47
HCB	1.6	0.01	0.03	0.02	0.06	0.02	0.09
Methoxychlor	NA	NA	NA	NA	NA	NA	NA

CSF: cancer slope factor.
NA: not available as there is no CSF value for methoxychlor.
Values in bold represent higher hazard ratio (>1.0).

and animal species. Thus, continuous monitoring studies to investigate the status of OCPs contamination in the Egyptian environment and food subjects are mandatory in Egypt.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors would like to thank Food Control Department, Faculty of Veterinary Medicine, Zagazig University for their kind support during conducting this study.

References

[1] P. J. John, N. Bakore, and P. Bhatnagar, "Assessment of organochlorine pesticide residue levels in dairy milk and buffalo milk from Jaipur City, Rajasthan, India," *Environment International*, vol. 26, no. 4, pp. 231–236, 2001.

[2] L. A. Thompson, W. S. Darwish, Y. Ikenaka, S. M. M. Nakayama, H. Mizukawa, and M. Ishizuka, "Organochlorine pesticide contamination of foods in Africa: Incidence and public health significance," *Journal of Veterinary Medical Science*, vol. 79, no. 4, pp. 751–764, 2017.

[3] N. P. Agnihotri, *"Pesticide safety evaluation and monitoring," All India coordinated research project on pesticide residues New Delhi*, Indian Agricultural Research Institute, New Delhi, India, 1999.

[4] Food and Agriculture Organization (FAO), *Joint FAO/WHO Food Standards Programme. Codex Alimentarious Pesticide residues in Food–Maximum Residue Limits*, Food and Agriculture Organization (FAO), 2nd edition, 2000.

[5] W. S. Darwish, Y. Ikenaka, W. R. El-Ghareeb, and M. Ishizuka, "High expression of the mRNA of cytochrome P450 and phase II enzymes in the lung and kidney tissues of cattle," *Animal*, vol. 4, no. 12, pp. 2023–2029, 2010.

[6] Y. B. Yohannes, Y. Ikenaka, S. M. M. Nakayama, A. Saengtienchai, K. Watanabe, and M. Ishizuka, "Organochlorine pesticides and heavy metals in fish from Lake Awassa, Ethiopia: insights from stable isotope analysis," *Chemosphere*, vol. 91, no. 6, pp. 857–863, 2013.

[7] Association of Official Agricultural Chemists (AOAC) International, "Official methods of analysis of AOAC International," P. Cunnif, Ed., Association of Official Agricultural Chemists (AOAC) International, Gaithersburg, Md, USA, 16th edition, 1999.

[8] United States Environment Protection Agency (USEPA)'s Integrated Risk Information System (USEPA IRIS), "United States Environment Protection Agency (USEPA)'s Integrated Risk Information System (USEPA IRIS)," 1998, https://www.epa.gov/sites/production/files/2015-06/documents/ny_hh_361_w_03121998.pdf.

[9] World Health Organization (WHO), *Dietary Exposure Assessment of Chemicals in Food (Chapter 6). Principles and methods for the risk assessment of chemicals in food. Environmental Health Criteria 240". FAO/WHO. International Programme on Chemical Safety (IPCS)*, World Health Organization (WHO), Geneva, Switzerland, 2009, http://www.who.int/ipcs/food/principles/en/index.html.

[10] FAO/WHO, "Pesticide Residues in Food. Joint FAO/WHO Meeting on Pesticide Residues. Report of the Joint meeting of the FAO Panel of Experts on PesticideResidues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. World Health Organization, Food and Agriculture Organization of the United Nations, Rome," 2009.

[11] Q. T. Jiang, T. K. M. Lee, K. Chen et al., "Human health risk assessment of organochlorines associated with fish consumption in a coastal city in China," *Environmental Pollution*, vol. 136, no. 1, pp. 155–165, 2005.

[12] M. C. Heck, J. Sifuentes dos Santos, S. Bogusz Junior, I. Costabeber, and T. Emanuelli, "Estimation of children exposure to organochlorine compounds through milk in Rio Grande do Sul, Brazil," *Food Chemistry*, vol. 102, no. 1, pp. 288–294, 2007.

[13] P. John, N. Bakore, and P. Bhatnagar, "Assessment of organochlorine pesticide residue levels in dairy milk and buffalo milk from Jaipur City, Rajasthan, India," *Environment International*, vol. 26, no. 4, pp. 231–236, 2001.

[14] G. Darko and S. O. Acquaah, "Levels of organochlorine pesticides residues in dairy products in Kumasi, Ghana," *Chemosphere*, vol. 71, no. 2, pp. 294–298, 2008.

[15] H. Deti, A. Hymete, A. A. Bekhit, A. M. I. Mohamed, and A. E.-D. A. Bekhit, "Persistent organochlorine pesticides residues in cow and goat milks collected from different regions of Ethiopia," *Chemosphere*, vol. 106, pp. 70–74, 2014.

[16] M. A. E.-H. Khairy, M. Kolb, A. R. Mostafa, A. EL-Fiky, and M. Bahadir, "Risk posed by chlorinated organic compounds in Abu Qir Bay, East Alexandria, Egypt," *Environmental Science and Pollution Research*, vol. 19, no. 3, pp. 794–811, 2012.

[17] V. Prachar, M. Veningerova, J. Uhank et al., "Persistent organochlorine compounds in cows milk and butter," *Fresenius Environmental Bulletin*, vol. 4, pp. 413–417, 1995.

[18] S. M. Waliszewski, R. Villalobos-Pietrini, S. Gómez-Arroyo, and R. M. Infanzón, "Persistent organochlorine pesticide levels in cow's milk samples from tropical regions of Mexico," *Food Additives & Contaminants: Part A*, vol. 20, no. 3, pp. 270–275, 2003.

[19] Y. Zhang, "Levels of organochlorine pesticide residues in food of China," *Pesticide Science and Management*, vol. 6, pp. 20–22, 1995.

[20] World Health Organization (WHO), *Health risks of persistent organic pollutants from long-range transboundary air pollution*, World Health Organization (WHO), 2003.

[21] E. M. Shaker and E. E. Elsharkawy, "Organochlorine and organophosphorus pesticide residues in raw buffalo milk from agroindustrial areas in Assiut, Egypt," *Environmental Toxicology and Pharmacology*, vol. 39, no. 1, pp. 433–440, 2015.

[22] D. Yahia and E. E. Elsharkawy, "Multi pesticide and PCB residues in Nile tilapia and catfish in Assiut city, Egypt," *Science of the Total Environment*, vol. 466-467, pp. 306–314, 2014.

[23] A. F. A. Mahmoud, W. S. Darwish, A. E. M. A. Morshdy, E. A. Eldaly, Y. Ikenaka, and M. Ishizuka, "Determination of organochlorine pesticides (OCPs) in the edible offal of Egyptian buffalo," *Japanese Journal of Veterinary Research*, vol. 61, pp. S58–S63, 2013.

[24] E. Kampire, B. T. Kiremire, S. A. Nyanzi, and M. Kishimba, "Organochlorine pesticide in fresh and pasteurized cow's milk

from Kampala markets," *Chemosphere*, vol. 84, no. 7, pp. 923–927, 2011.

[25] K. A. Hassal, *Biochemistry and Uses of Pesticides*, Macmillan Press Ltd., 1990.

[26] A. F. A. Mahmoud, Y. Ikenaka, Y. B. Yohannes et al., "Distribution and health risk assessment of organochlorine pesticides (OCPs) residue in edible cattle tissues from northeastern part of Egypt: High accumulation level of OCPs in tongue," *Chemosphere*, vol. 144, pp. 1365–1371, 2016.

[27] "ATSDR (Agency for Toxic Substances and Disease Registry), "Toxicological Profiles of Toxic Substances. US Department of Health and Human Services. Public Health Service, Atlanta, GA", 2005," 2005, http://www.atsdr.cdc.gov.

[28] V. Pardío, D. Martínez, A. Flores et al., "Human health risk of dietary intake of organochlorine pesticide residues in bovine meat and tissues from Veracruz, México," *Food Chemistry*, vol. 135, no. 3, pp. 1873–1893, 2012.

[29] R. Zhou, L. Zhu, and Q. Kong, "Persistent chlorinated pesticides in fish species from Qiantang River in East China," *Chemosphere*, vol. 68, no. 5, pp. 838–847, 2007.

[30] R. McKinlay, J. A. Plant, J. N. B. Bell, and N. Voulvoulis, "Calculating human exposure to endocrine disrupting pesticides via agricultural and non-agricultural exposure routes," *Science of the Total Environment*, vol. 398, no. 1-3, pp. 1–12, 2008.

[31] G. C. Windham, D. Lee, P. Mitchell, M. Anderson, M. Petreas, and B. Lasley, "Exposure to organochlorine compounds and effects on ovarian function," *Epidemiology*, vol. 16, no. 2, pp. 182–190, 2005.

[32] R. W. Bretveld, M. Hooiveld, G. A. Zielhuis, A. Pellegrino, I. A. L. M. van Rooij, and N. Roeleveld, "Reproductive disorders among male and female greenhouse workers," *Reproductive Toxicology*, vol. 25, no. 1, pp. 107–114, 2008.