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Risk assessment of pesticides and heavy metals contaminants in vegetables: A novel bioassay method using *Daphnia magna* Straus

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ABSTRACT

Cucumber and potato samples of known levels of pesticides and heavy metal residues, as respectively measured by gas chromatography and atomic absorption, were subjected to a bioassay method using *Daphnia magna* in order to assess the potential of the toxic hazard of their contaminants. Based on the estimated lethal time for 50% mortality (LT50) in daphnids, we suggested a classification to categorize toxic hazards in six definite ratings. Either samples of cucumbers (from conventional, greenhouse and organic farming) or potatoes (from conventional and organic farming) were evaluated for toxic hazard of the mixtures of pesticide residues and heavy metals, as well as mixtures of both. Accordingly, a 53.7% of cucumber samples were ranked as "Highly Toxic: HT"; a 18.5% "Moderately Toxic: MT); a 9.3% "Slightly Toxic: ST"; and a 18.5% "Practically Non-Toxic: NT". For potato samples, the ranking pattern to different classes was: Extremely Toxic: ET (LT50 = <1 h) for 11.1%; Very Toxic: VT (LT50 = 1–<3 h) for 50.0%; HT (LT50 = 3–<12 h) for 13.9%; MT (LT50 = 12–<24 h) for 11.1%; ST (LT50 = 24–48 h) for 0.0%; and NT (LT50 = > 48 h) for 13.9% of the samples bioassayed.

1. Introduction

The environmental analysis helps to protect the natural environment and human health through testing contaminants, such as pesticides, metals and other hazardous toxins and pollutants in air, water, soil and food. Food safety testing includes the analysis of agricultural products and foods, with a focus on regulatory compliance and enforcement. This rapidly growing issue is being driven by the liberalization of global trade, an increasingly stringent regulatory environment and heightened public awareness of food safety issues. One of the principal applications of toxicology data is to inform risk assessments and support risk management decisions that are protective of human health. Ideally, a risk assessor would have available all of the relevant information on (a) the toxicity profile of the agent of interest; (b) its interactions with living systems; and (c) the known or projected exposure scenarios: to whom, how much, by which route(s), and how often. In practice, however, complete information is seldom available. Nonetheless, decisions still must be made (Doull et al., 2007).

Assessment and control of environmental chemical pollutants have traditionally been dependent upon physico-chemical monitoring to identify and quantify toxicants and to provide data which, for regulatory purposes, could be compared to allowable concentrations for a particular receiving environmental samples. Although great advances have been made in the design and accuracy of chemical sensors and associated analytical procedures, such monitoring techniques are inadequate on several counts. Therefore, a realistic interpretation of chemical toxicity to biological systems can only be carried out by use of a system which actually employs living organisms (Pascoe, 1987). Also, It has been long recognized that the chemical characterization of hazardous substances in an environmental matrix such as wastes, is not a practical approach in the majority of the cases due to the presence of complex mixtures of chemicals; and for that reason, ecotoxicological testing may be a complementary pragmatic approach to the chemical characterization for the management of complex wastes (CEN, 2006; Leitgib et al., 2007; Berthelot et al., 2008; Wilke et al., 2008; Pablos et al., 2009). These led scientists to develop biological monitoring programs alongside and are complementary to physico-chemical monitoring.

Environmental samples are usually polluted with a variety of compounds and thus represent complex situations in terms of toxicity assessment. It is uncommon to find an aquatic or other environmental system which is polluted by a single toxicant, and usually several harmful substances are present together in it; leading to possible interactions between such pollutants and between their effects on the tested organisms (van Leeuwen and Hermens, 1988). On the other hand, exposure to environmental chemicals is unintentional, and often the exposure is to chemical mixtures,

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either simultaneously or sequentially. When exposure occurs, in public health practice, it is prudent to ascertain if thresholds for harmful health effects are exceeded, whether by individual chemicals or by chemicals in combination. Three alternative approaches are available for assessing the toxicity of chemical mixtures. Each approach, however, has shortcomings. Recently, reliance has increased on computational toxicology methods for predicting toxicological effects when data are limited. Advances in molecular biology, identification of biomarkers, and availability of accurate and sensitive methods allow us to more precisely define the relationships between multiple chemical exposures and health effects, both qualitatively and quantitatively (Mumtaz et al., 2007).

Toxicity testing has grown steadily in recent years, being a useful tool in environmental risk assessment. Different bioassay methods and recently developed biosensors based on acute toxicity measurements have been developed with emphasis placed on the use of combined approaches involving chemical analysis for the characterization and identification of complex toxic substances in wastewater effluents, sewage sludge and other environmental samples within bioassay-directed chemical-analysis protocols (Farré and Barceló, 2003).

Seco et al. (2003) concluded that ecotoxicity tests may also be used, as chemical tests are, for the assessment of solid waste treatment, but a better result may be obtained by the complementary use of both ecotoxicological and chemical tests. Several studies have been directed to combine between ecotoxicity of industrial wastes containing potentially toxic substances (e.g., heavy metals) and the chemical composition estimation of these wastes. Ecotoxicity based on *Daphnia magns* acute toxicity has been extensively used in this context (e.g., Lambolez et al., 1994; Ortiz et al., 1995; Kaneko, 1996; Seco et al., 2003).

In the field of toxicant bioassay, the freshwater cladoceran *Daphnia magna* Straus is one of the oldest and widely used test organisms in aquatic toxicology (Baudo, 1987). The genus *Daphnia* is an important link in freshwater trophic chains as dominant consumer of primary producers and as food for both invertebrate and vertebrate predators. Most species of this genus are used as test organisms representing the filter-feeding zooplankton (Mark and Solbe, 1998). The choice of *D. magna* as standard test species was influenced by several advantageous characteristics. It is of small size and easy to culture in the laboratory. Its parthenogenetic reproduction under non-stressed conditions allows the testing of clones, which enhances the reproducibility and repeatability of the test results. Furthermore the organism is relatively sensitive to chemicals compared with other freshwater invertebrates (e.g., Versteeg et al., 1997; Mark and Solbe, 1998), and its relatively short life-span and reproduction cycle are favorable for the chronic testing. Thus *D. magna* is the most commonly tested freshwater species in acute as well as in chronic tests (Ratte and Hammerswirtz, 2003).

In the field of chemical monitoring of environmental pollutants, gas chromatographic (GC) techniques are widely used in identification and quantification of organic pollutants, such as pesticides, while atomic absorption spectrophotometric (AAS) tools are used for metal analyses. Determination of pesticide residues in foods, such as vegetables, goes through subsequent steps ending with taking the pesticide residues from the plant tissue into a small volume of an organic solvent to be injected into the GC apparatus. In case of AAS analyses, the metals are extracted in an aliquot solution after sample's digestion by a strong acid. Usually, very few micro liters from the final solution are utilized in the analyses and the whole bulk neglected. The method investigated in the present study is aiming at taking benefit of using such final solutions in bioassaying potential toxicity of their already known contaminants by using the water flea, *D. magna* Straus in a "Bioassay-directed chemical-analysis protocol".

2. Materials and methods

2.1. Experimental animals

A single laboratory colony of *D. magna* Straus cultured in the laboratory since 1988 (Mansour et al., 1992) was used in this study. Bulk cultures of 15 animals each were maintained in ASTM hard synthetic water (Barata et al., 2000) and the animals were fed daily yeast suspension (Mansour et al., 1993). The cultures were used to obtain neonates of <24 h according to the method described by Dewey and Parker (1964), then maintained in the culture room at 20 ± 2 °C and 12:12 h light: dark cycle. At the 9th day, the grown female adults (9–10 d-old) were harvested and used in the bioassays conducted in the present study.

2.2. Test solutions

In previous studies, we determined pesticide residues and heavy metals in conventional (C), greenhouse (G) and organic (O) cucumber fruit samples (Mansour et al., 2009a), as well as in (C) and (O) potato tuber samples (Mansour et al., 2009b). The samples were monthly collected during June 2006–May 2007 from the local market in Cairo, Egypt and subjected to gas chromatographic (GC) and atomic absorption spectrophotometric (AAS) analyses. Quality control assurance criteria (e.g., detection and determination limits, recovery, and reproducibility) were evaluated for each analyzed pesticide and metal before running sample analyses (data are presented in Mansour et al., 2009a,b). Test tubes containing residual film of pesticide residues and glass bottles containing ca. 25 ml aliquots of heavy metals mixture similar to those subjected to final measurement steps were kept in a deep freezer (−20 °C) for bioassay purposes planned to the present investigation.

2.3. Bioassay

The experimental protocol for evaluating acute toxicity of the concerned toxicants was run as illustrated in Fig. 1. To set up this protocol, preliminary experiments were carried out to test suitable dilution for the original stock samples in which ca. 75% of daphnids can survive up to 1 h exposure time. To ensure repeatability, a number of final working solutions were bioassayed two times. For pesticides mixture bioassay, the residual film of pesticide residues was reconstituted in 1 ml dimethyl sulfoxide (DMSO) and 0.5 ml was pipette into a 10 cm petri dish containing 50 ml deionized water and 10 daphnids adult. Control test contained the same amount of DMSO. Observations on *Daphnia* were recorded 1, 2, 3 and 24 h after exposure.

For heavy metals mixture bioassay, the volume of the solution in the bottle was accurately measured and doubled by deionized water, then a 0.5 ml was pipette into a 10 cm petri dish containing 50 ml deionized water and 10 daphnids adult. Control test was prepared by adding 0.01 ml of concentrated HCl to the deionized water in the petri dish before placing daphnids. This amount of HCl is supposed to proportionate the acid which was used in the sample digestion process. Observations on *Daphnia* were recorded 1, 2, 3 and 24 h after exposure.

For bioassaying both mixtures of pesticides and heavy metals in one test container, 0.5 ml of the test tube solution (pesticides) plus 0.5 ml of the bottle solution (heavy metals) were transferred into a petri dish containing 50 ml deionized water and 10 daphnids. Control test contained 0.5 ml DMSO + 0.01 ml of concentrated HCl. Observations on *Daphnia* were recorded 1, 2, 3 and 24 h after exposure.

All experiments were carried out in duplicate, and in all cases the final test solution in each petri dish was adjusted to 50 ml after placing the daphnids. The test containers were placed on black sheets to facilitate observations on daphnids and incubated at room conditions (20 ± 2 °C; 70 ± 5%RH).

2.4. Criteria of assessment

Both complete immobilization (death) and any of symptomatic responses (e.g., circular rotary motion, somersaults, floating on the surface or sinking to the bottom of the test solution, partial paralysis with occasional antenna movement, etc.), previously described by Parker et al. (1970), were used as toxicity criteria for the bioassayed samples. Mortality percentages versus time in hours were plotted on semi-logarithmic – probit scale using a software Ld-P line program, based on Finney (1971), to estimate lethal time for 50% mortality in daphnids exposed to the different test solutions (LT50s).

3. Results

Out of the monthly-12 samples subjected to instrumental physico-chemical analyses in our previous work (Mansour et al., 2009a,b); samples representing 6 months were selected for the present investigation. These were of August, September, October, December, 2006, February, and May, 2007 (for cucumbers), and of August, September, November, 2006, January, February, and