Mutation Research 770 (2016) 183–203

Contents lists available at ScienceDirect

Mutation Research/Reviews in Mutation Research

journal homepage: www.elsevier.com/locate/reviewsmr
Community address: www.elsevier.com/locate/mutres

Review

The use of the lymphocyte cytokinesis-block micronucleus assay for monitoring pesticide-exposed populations

Claudia Bolognesi[a,*], Nina Holland[b]

[a] Environmental Carcinogenesis Unit, IRCCS Azienda Ospedaliera Universitaria San Martino-IST Istituto Nazionale Ricerca sul Cancro, L.go Rosanna Benzi 10, Genoa, Italy
[b] School of Public Health, University of California, Berkeley, CA, USA

ARTICLE INFO

Article history:
Received 16 December 2015
Received in revised form 21 April 2016
Accepted 22 April 2016
Available online 26 May 2016

Keywords:
Lymphocyte cytokinesis-block micronucleus
Pesticide
Biomonitoring
Genotoxicity
Systematic review

ABSTRACT

Pesticides are widely used around the world, and hundreds of millions of people are exposed annually in occupational and environmental settings. Numerous studies have demonstrated relationships between pesticide exposure and increased risk of cancers, neurodegenerative and neurodevelopmental disorders, respiratory diseases and diabetes. Assessment of genotoxicity of pesticides and biomonitoring their effect in exposed populations is critical for a better regulation and protection, but it can be complicated because pesticides are often used as complex mixtures. The cytokinesis-block micronucleus assay in human lymphocytes (L-CBMN) is a validated method of assessment of DNA damage induced by clastogenic and aneuploidogenic mechanisms. The goal of this review is to provide an updated summary of publications on biomonitoring studies using this assay in people exposed to pesticides in different settings, and to identify gaps in knowledge, and future directions. A literature search was conducted through MedLine/PubMed and TOXLINE electronic databases up to December 2015. A total of 55 full-text articles, related to 49 studies, excluding reviews, were selected for in depth analysis, divided by the settings where exposures occurred, such as chemical plant workers, pesticide sprayers, floriculturists, agricultural workers and non-occupationally exposed groups. Majority of studies (36 out of 49) reported positive findings with L-CBMN assay. However, most of the studies of professional applicators that used single pesticide or few compounds in the framework of specific programs did not show significant increases in MN frequency. A decreased level of pesticide-induced genotoxicity was associated with the proper use of personal protection. In contrast, subjects working in greenhouses or during intensive spraying season and having acute exposure, showed consistent increases in MN frequency. Overall, this analysis confirmed that L-CBMN is an excellent tool for pesticide biomonitoring, and can validate the effects of educational and intervention programs on reducing exposure and genetic damage.

Contents

* Corresponding author.
E-mail addresses: claudia.bolognesi@hsanmartino.it, claudiabolognesi@yahoo.it (C. Bolognesi).

http://dx.doi.org/10.1016/j.mrrev.2016.04.006
1383-5742/

1. Introduction

A “pesticide” is defined as any substance or mixture of substances used to kill, repel or control a “pest”, including, fungi, bacteria, insects, snails, worms, rodents and weeds. This general term covers specific groups of agrochemicals such as herbicides, fungicides, insecticides, acaricides, nematicides, molluscicides, rodenticides, growth regulators, repellents, rodenticides and biocides [1]. Pesticides are biologically active compounds designed to selectively affect functional systems or target molecules specific to the “pest”. Due to the similarities in the biological macromolecules of all organisms an absolute selectivity is difficult to achieve, therefore the large majority of pesticides is characterized by various degree of toxicity to non-target species, including humans. Pesticides have multiple uses: they are applied to protect crops from insects, weeds and fungal diseases while they are growing, and to prevent food contamination by rats, mice, insects and fungi during storage. Pesticides are also used to control vector-borne diseases and noxious insects and to protect animals from illnesses that can be caused by parasites and fleas. Herbicides are employed to clear roadside weeds, trees and brush and are commonly applied in ponds and lakes to control algae and plants such as water grasses that can interfere with swimming and fishing.

The Pesticide Manual lists 10,400 product names associated with 1630 substances belonging to different chemical classes that are, or have been, used as pesticides [2]. The active ingredients are formulated in a variety of ways, such as liquids, dusts, granules, impregnated pellet-tablets, resin strips and concentrates. The formulations also contain a number of “inert” ingredients, such as solvents and surfactants that play an important role in the effectiveness of a pesticide product. These additives may have toxic effects different from the active ingredients.

Pesticides are used worldwide. The general population can be exposed to low concentrations of agricultural pesticides through contamination of air, water, food supplies [3], and also through household use [4]. High exposures are associated with the production, packaging and application of these compounds in agriculture or for purposes of protection of public health such as malaria prevention. Three main routes are relevant for the exposure to pesticides: inhalation by breathing air containing pesticide aerosol or small particles with adsorbed pesticide, dermal absorption during scattering, mixing and loading of powders and liquids, spraying and crop harvesting, and oral ingestion of contaminated food or water.

Acute pesticide poisoning, associated with specific chemical classes or compounds is a major public health problem, mainly in developing countries. Each year, at least 300 million people worldwide are estimated to suffer from acute pesticide poisoning [5,6].

A number of pesticides have been characterized as possible or probable human carcinogens by IARC based on human and experimental animal data showing links between some pesticides and cancer at multiple sites [7]. Cancers of the lung, prostate, lymphatic and hematopoietic system are most frequently associated with pesticide exposure in epidemiological studies [8,9]. For example, pesticides from different chemical and functional classes were related with an excess risk of non-Hodgkin lymphoma [10]. Moreover, a recent study suggests that occupational maternal pesticide exposure during pregnancy, and paternal exposure prior to conception, may increase the risk of leukemia in the offspring [11].

Epidemiological findings, along with experimental laboratory data, reveal a positive association between pesticide exposure and different neurodegenerative disorders such as Parkinson’s disease (PD) [12], Alzheimer’s disease (AD) [13], amyotrophic lateral sclerosis (ALS) [14]. Other studies demonstrated that exposure to pesticides may cause other diseases or health effects, such as neurodevelopmental toxicity [15], non-malignant respiratory diseases [16], diabetes [17] and immune toxicity [18]. A number of studies suggest that an interaction between certain gene polymorphisms and exposure to pesticide increases the risk for PD [19], adverse birth outcomes [20], and neurodevelopmental problems [21–23].

Pesticides can exert their toxic effects to humans through various modes of action either related or different from their mechanism of action against the target “pest” [24]. The genotoxic potential of pesticides has been extensively studied as a primary risk factor for long-term health effects such as cancer and degenerative diseases. The majority of pesticides in use have been tested in a wide variety of mutagenicity assays that cover gene mutations, chromosomal alterations, and DNA damage. Experimental data revealed that many agrochemical substances are mutagenic, and induce different genetic endpoints [25]. As a consequence, many pesticides were banned from the market or restricted in use for their carcinogenic and/or genotoxic properties, but due to their bioaccumulation potential, could persist in environment for years.

Current regulations concerning the introduction of pesticides to the market (e.g. Dir. 91/414/EEC; US-EPA regulations) involve the evaluation of the active substances in a comprehensive number of tests in different biological systems *in vitro* and *in vivo* and, do not allow the use of pesticide formulations containing carcinogenic and/or genotoxic components. However, the exclusion of genotoxic potential for single compounds introduced in the market as pesticides may not prevent long-term risk for humans associated with the practical use of agrochemical formulations (including inert ingredients) possibly interacting with each other.

The biomonitoring of genotoxicity in human populations is a useful tool to estimate the exposure and potential risk from an integrated exposure to complex mixtures of chemicals. Biomonitoring studies in different countries have been carried out to elucidate the risk associated to the exposure to specific compounds and pesticide-related occupations, or to specific cultivation practices.

The aim of this review is to retrieve, analyze and summarize published studies that used the lymphocyte cytokinesis-block micronucleus (L-CBMN) in pesticide exposed subjects to:

- Confirm L-CBMN as an informative biomarker associated with exposure to pesticides
- Assess the genotoxicity of pesticides in specific occupational and environmental settings