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Effect of safeners on damage of human erythrocytes treated with chloroacetamide herbicides

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ABSTRACT

Chloroacetamides are used as pre-emergent substances for growth control of annual grasses and weeds. Since they can be harmful for crop plants, protective compounds (safeners) are used along with herbicides. So far, their effects on human blood cells have not been evaluated, and this study is the very first one devoted to this subject.

We examined the harmful effects of chloroacetamides, their metabolites and safeners, used alone or in combination with herbicides, on human erythrocytes measuring the extent of hemolysis, lipid peroxidation and catalase activity. Higher impact of herbicides than their metabolites on all of the investigated parameters was found. Safeners alone did not produce any damage to erythrocytes and did not elicit any changes in oxidative stress parameters. Combination of safener with herbicide did not attenuate hemolysis of erythrocytes compared to the herbicide alone. Safeners reduced lipid peroxidation induced by herbicides, which suggest the role of safeners as antioxidants.

1. Introduction

Herbicides are a diverse group of pesticides used worldwide in weed control and management. So far, their application is still the most effective and customary procedure for plant protection and has contributed significantly to the enhancement of agricultural productivity and crop yields. In many countries, e.g. USA, the amount of herbicides is increasing while in European agriculture their usage decreases. This is partly attributed to a shift toward organic and manual weed control methods, which are better suited for the environment. A number of European countries, such as France, Germany, Spain, Norway, Italy and the United Kingdom, however, still rely on herbicides to manage weed growth for commercial crops (Rational Herbicide Use, 2012). As a consequence, the

Abbreviations: ABC transporter, ATP-binding cassette transporter; CD, conjugated dienes; CDEPA, 2-chloro-N-(2,6-diethylphenyl)acetamide; CMEPA, 2-chloro-N-(2-methyl-6 ethylphenyl)acetamide; DEA, 2,6-diethylaniline; GSH, glutathion; GST, glutathione-S-transferase; LPO, lipid peroxidation; MDA, malondialdehyde; MDR1, multidrug resistance transporter; MEA, 2-methyl-6-ethylaniline; MEL, melatonin; MRP1, multidrug-resistance protein; NAC, N-acetyl-L-cysteine; ROS, reactive oxygen species; TBA, thiobarbituric acid; TBARS, thiobarbituric acid-reactive substances; TCA, trichloroacetic acid; TSH, thyroid-stimulating hormone; UDPGT, UDP-glucuronosyltransferase; USEPA, United States Environmental Protection Agency.

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general population is inevitably exposed to the residues of these compounds, including physical and biological degradation products in air, water and food.

Taking into account that pesticides do not act selectively it can be stated that most of them are also toxic to crop plants, animals and humans the more so that over 60% of the poundage of all agricultural herbicides consist of those that are capable of disrupting the endocrine and/or reproductive systems of animals (Colborn and Short, 1999). Occupational hazard occurring at all stages of pesticide formulation, manufacture and implementation, involves exposure to complex mixtures of different types of chemicals, active ingredients and by-products present in technical formulations, such as impurities, solvents and other compounds produced during the storage procedure (Bolognesi, 2003). The prevalence of these chemicals in the environment has stimulated investigations on the degradation of hazardous substances in water and contaminated soil.

Ones of the most exploited herbicides in agriculture are chloroacetamides, used for major crops including corn, soybeans, sorghum, and rice (Jablonkai, 2003). This class of pesticides consists of a number of broad-spectrum, pre-emergence herbicides such as alachlor, acetochlor, amidochlor, butachlor, metolachlor and propachlor. Residues of alachlor and acetochlor are reported in groundwater, surface water and soil (Dagnac et al., 2002; Gan et al., 1995; Kidwell, 2007; Kolpin et al., 1996; Visi et al., 1998). It is, therefore, necessary to consider possible mechanisms for toxicity and genotoxicity of these plant protection products toward humans.

It has been found that pesticides could be detrimental for human health (Colborn, 2006). U.S. Environmental Protection Agency (USEPA) classified alachlor and acetochlor as probable human carcinogens. Alachlor use is banned in European Union countries because of its mutagenicity and potential carcinogenicity. In other places e.g. United States, South America and Asian countries, use of alachlor is still unrestricted, even though it has been proven to induce lung tumors in mice and stomach and thyroid and nasal turbinate tumors in rats (USEPA, 1998a). Acetochlor has been reported as a potential human carcinogen, as well (Kidwell, 2007).

It has been suggested that the carcinogenicity of these compounds involves a complex metabolic activation pathway leading to a DNA-reactive dialkylbenzoquinone imine. Important intermediates in this pathway are 2-chloro-N-(2,6-diethylphenyl)acetamide (CDEPA) produced from alachlor and 2-chloro-N-(2-methyl-6-ethylphenyl)acetamide (CMEPA) produced from acetochlor. Subsequent metabolism of CDEPA and CMEPA produces 2,6-diethylaniline (DEA) and 2-methyl-6-ethylaniline (MEA), which are bioactivated through para-hydroxylation and following oxidation to the proposed carcinogenic product dialkylbenzoquinone imine (Coleman et al., 2000; Kale et al., 2008). Alachlor and acetochlor are metabolized by CYPs 2B6 and 3A4 (Hodgson, 2001), while the liver microsomal arylamidase is responsible for the metabolism of both CDEPA and CMEPA. Furthermore, the key metabolites of chloroacetamides, such as quinonimine, have been found to promote the formation of reactive oxygen species (ROS) (Bagchi et al., 1995), which are involved in induction of DNA damage (Bonfanti et al., 1992) and modification of cell death. Dialkylquinonimine metabolites of chloroacetamide herbicides also induce genotoxic effects in cultured human lymphocytes, such as sister chromatid exchanges (Hill et al., 1997), chromosomal aberrations (Ashby et al., 1996) and micronuclei formation (Ateeq et al., 2005).

To protect crop plants from herbicide damage without reducing their activity in target weeds new compounds, safeners, also known as antidotes, have been introduced. Herbicide safeners are used commercially and can be applied either as a mixture together with the herbicide or individually as a seed-pretreatment prior to sowing (Davies, 2001). One of the most widely used safeners are dichloroacetamides, e.g. dichlormid, which are particularly effective in protecting maize and sorghum against chloroacetamide herbicides (Walton and Casida, 1995). Employment of safeners similar in structure to the herbicide has been proposed as the most effective as safeners are believed to compete with herbicide molecules for the binding sites on the target proteins (Bordas et al., 2000; Sephenson and Chang, 1978).

There is no conclusive mechanism explaining mechanisms of action of the available safeners. Various hypotheses have been proposed such as interference with uptake and translocation of the herbicide, variations in herbicide metabolism and competition at the site of action of the herbicide. The most likely mechanism of action of safeners seems to be an improvement of herbicide metabolism. Conjugation of herbicides with glutathione (GSH) is a common pathway of their metabolism thus safeners could increase crop tolerance to herbicides through an increase of GSH content (Farago et al., 1994), regulation of the expression of genes involved in herbicide metabolism and an increase of the level of messenger RNA encoding glutathione-S-transferase (GST), responsible for herbicide conjugation (Jepson et al., 1994; Wiegand et al., 1986). The newest hypothesis resulting from the most recent research assumes that safeners may be utilizing an oxidized lipid-mediated (oxylipins) or cyclopentenone-mediated signaling pathway, leading to the expression of GSTs and other proteins involved in detoxification and plant defense (Mosblech et al., 2009; Mueller et al., 2008; Mueller and Berger, 2009; Riechers et al., 2010).

There are no available data on the human toxicity of safeners though it has been reported that metabolism of dichlormid in mammals can be similar to that demonstrated in plant and soil (USEPA, 1998b). Addition of inert ingredients, like safeners, to the herbicide mixture may lead to biological activation and synergistic effects. Having this knowledge it is necessary to examine the effects of the combination of herbicides and safeners on the human organism.

In this study, we aimed at investigating the harmful effects of safeners and chloroacetamide herbicides, used alone or in combination, on human red blood cells. Pyrazoline (mefenpyr) and dichloroacetamide (dichlormid) safeners and herbicides alachlor and acetochlor, as well as their metabolites DEA and MEA, have been investigated. A degree of erythrocyte hemolysis and changes in some of the parameters of intracellular oxidative stress were estimated as an indicator of erythrocyte damage after exposure to investigated compounds. It should be emphasized that so far the detrimental effects of safeners, used alone or in conjunction with herbicides, on human erythrocytes have not been investigated by other authors, and