Detection of cellular damage after exposure at not-cytotoxic doses of chlorpyriphos solution by Raman microspectroscopy and AFM

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Among the chemical products used for agricultural activities, Chlorpyriphos has an important role because it exhibits insecticidal activity against a wide range of insects on foliage in a wide range of crops [1]. The study of the effetcs of Chlorpyriphos exposure of workers in the manufacturing and utilization activities as well as consumers are important to protect humans and their habitat. In particular, studies of exposed cultured cells are essential for the investigation of the biological response to pesticides at cellular level, in order to evaluate eventual biochemical and structural modifications caused by such products, especially when low doses exposure occurs. Raman microspectroscopy (RM) has been widely used in the last years for analysing molecular composition of single cells exposed to chemical stress with micrometric spatial resolution [2,3]. Also Atomic Force Microscopy (AFM) has been extensively used to investigate morphological modifications in cells exposed to toxic agents [4,5]. Therefore, both such techniques are suited to investigate the damage induced by exposure to pesticides, such as Chlorpyriphos, at cellular level. The scope of this contribution is to investigate chemical and morphological modifications in normal human keratinocytes cells exposed to chlorpyriphos solution at different concentrations by means of RM and AFM techniques. The choice of such cells is related to the fact that keratinocytes are the human cells most exposed to the environmental stress.

Normal human keratinocyte (HUKE) cells were treated with increasing doses of chlorpyriphos for 24h at concentrations from 10^{-3} M to 10^{-6} M. Trypan blue viability test indicated that exposure to 10^{-3} M of chlorpyriphos for 24 h can be considered as a cytotoxic dose. The samples measured by RM and AFM consisted of cells cultured on poly-lysine coated glass and fixed in paraformaldehyde.

Biochemical modifications of keratinocytes exposed to different concentrations of chlorpyriphos solution were detected by means of RM, as shown in Fig 1. The modifications mainly consist of breakdown of amide linkage between aminoacids (amide I and amide III peaks). Damage of DNA bases and single aminoacids occurs as well. The protein and DNA modifications begins after exposure at chlorpyriphos concentration value (10^{-6} M) at least three orders of magnitude lower than the concentration value estimated as cytotoxic (10^{-3} M).

In contrast to Raman measurements, AFM images have revealed that the Chlorpyriphos treatment at not cytotoxic doses scarcely influence the morphology of the plasmatic membrane of keratinocytes. In fact, the images in Fig. 2 show that the untreated cells are characterized by a smooth surface, without roughness and holes, as can be also deduced from the cross-section of topographic profile. The images of the treated cells are also similar to the control one as for the regular shape, but the cross sections of topographic profile show that the surfaces of the treated cells are slightly rougher with respect to the surface of control cells.

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Therefore, the exposure of keratinocytes at chemical solutions containing Chlorpyriphos with concentration value well below the cytotoxic value causes a partial morphological damage to the plasmatic membrane and strongly affect the protein and DNA structure.

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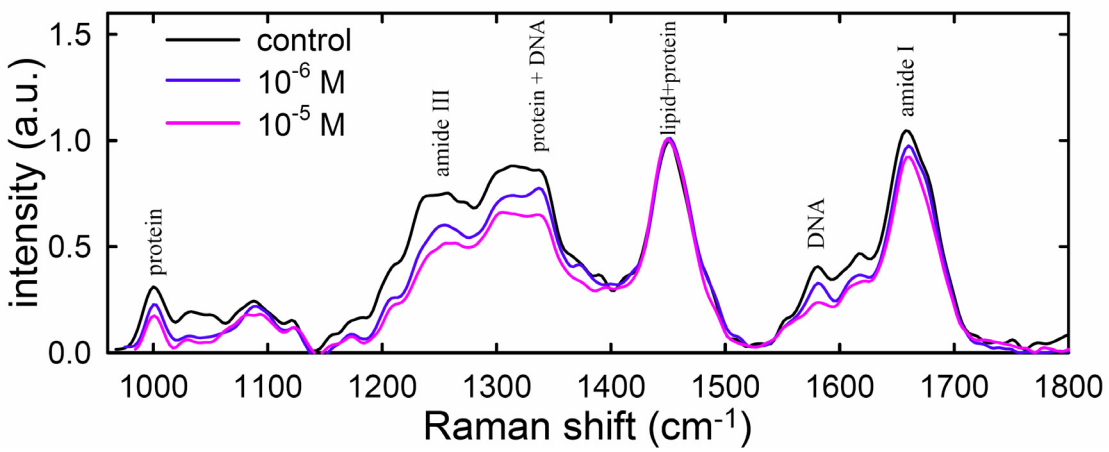

Figure 1. Raman spectra of control and chlorpyriphos exposed keratinocytes. The different concentrations of chlopyriphos, at which the cells have been exposed, are indicated on the left hand side. Each spectrum has been averaged over Raman spectra measured on ten keratinocyte cells randomly chosen and has been normalised to the peak intensity of the 1450 cm^{-1} feature.

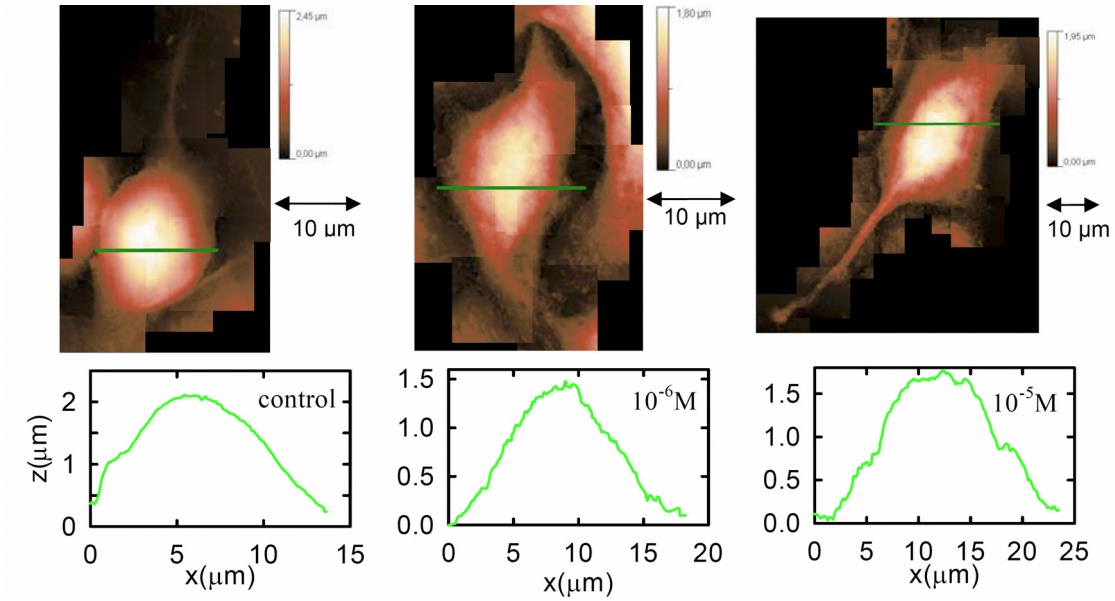

Figure 2. AFM images in contact mode topography and cross sections of typical control and exposed keratinocytes. The Chlorpyriphos concentration in the exposure solution is reported in the cross-section plot of each image. The scale bar is reported on the right hand side of each image.

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