The use of pesticides has been continuously increasing worldwide since their introduction in the global market, with the aim of fulfilling the needs of the growing population. While their monitoring in the environment has been thoroughly addressed, the bioaccumulation of pesticides in the human body is an ongoing challenge, due to the complexity of the biological matrices in which pesticides tend to accumulate (e.g., urine, blood, sweat, and more). Most of the developed strategies rely on time-consuming sample preparation or expensive and polluting reagents for the construction of the sensing platform. In the view of developing a green alternative device to handle pesticide analysis in biofluids, we designed an enzymatic paper-based electrochemical sensor for glyphosate (GLY) detection in human urine. The pesticide quantification is obtained by measuring the grade of enzyme inhibition by both GLY and uric acid (UA), the latter being one of the principal components of human urine. GLY detection is carried out by measuring the initial and residual enzymatic activity in chronoamperometric tests, taking into consideration the contribution of UA to the inhibition. In addition to the specific application here described, this work aims at providing an emblematic example of how to design green sensing solutions for addressing analytical challenges related to health control, delivering low-impact methods based on sustainable materials (e.g., paper), non-toxic reagents, and minimal waste production while ensuring competitive analytical performances.