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Título artículo: Determination of glyphosate exposure in the Iberian hare: a potential focal species associated to agrosystems

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RESUMEN: Glyphosate is the most used herbicide worldwide. It is a small and highly polar pesticide whose physicochemical properties makes its analytical determination difficult. Here, a procedure based on liquid chromatography-high resolution tandem mass spectrometry (LC-HRMS/MS) was developed for glyphosate determination in samples of gastric content from wildlife. Iberian hare (*Lepus granatensis*), a herbivorous mammal species, strongly associated to agrosystems was selected as model species. The procedure involves direct analysis of sample without derivatization or instead of neither further cleaning steps. The procedure was validated by inter-day accuracy and precision studies with gastric content of hare spiked with glyphosate at ecologically relevant concentrations for the species (0.1-6 μg/g), and with 1 μg/g of isotopically labelled internal standard (glyphosate-2-13C,15N). Finally, glyphosate residues in hunted animals from pesticide-treated and pesticide-free areas (n=75 and 28, respectively), as well as from hares found dead in the field (n=11) were analysed. The linearity of both standards in extraction solutions and procedural calibration curves with spiked samples was similar, both with determination coefficients (r2) higher than 0.99. Satisfactory recoveries in spiked samples were achieved within the range of 95% to 118% (CV ≤ 20%). The limit of detection of glyphosate in hare gastric content was 0.03 μg/g. Prevalence of glyphosate in hunted animals from pesticide-treated areas ranged between 9-22%, increasing to 45% in animals found dead. The glyphosate concentrations detected in the gastric content of hares ranged from 0.11 to 16 μg/g. No residues were detected in animals from pesticide-free areas. In practice, the developed methodology may be particularly useful in the context of research and other work on the exposure in wildlife of one of the most used pesticides nowadays.

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