A novel electrochemical immunosensing strategy for the detection of atrazine based on magnetic beads is presented. Different coupling strategies for the modification of the magnetic beads with the specific anti-atrazine antibody have been developed. The immunological reaction for the detection of atrazine performed on the magnetic bead is based on a direct competitive assay using a peroxidase (HRP) tracer as the enzymatic label. After the immunochemical reactions, the modified magnetic beads can be easily captured by a magnetosensor made of graphite–epoxy composite, which is also used as the transducer for the electrochemical immunosensing. The electrochemical detection is thus achieved through a suitable substrate and mediator for the enzyme HRP. The electrochemical approach is also compared with a novel magneto-ELISA based on optical detection. The performance of the electrochemical immunosensing strategy based on magnetic beads was successfully evaluated using spiked real orange juice samples. The detection limit for atrazine using the competitive electrochemical magnetoimmunosensing strategy with anti-atrazine-specific antibody covalent coupled with tosyl-activated magnetic beads was found to be $6 \times 10^{-3} \mu g L^{-1} (0.027 \text{ nmol L}^{-1})$. This strategy offers great promise for rapid, simple, cost-effective, and on-site analysis of biological, food, and environmental samples.

Pesticides represent a major hazard to public health throughout the world, due to the sheer volume of pesticide usage, coupled to their universal distribution, persistence in the environment, and toxicological properties. Atrazine has been one of the most widely used herbicides for the selective control of annual grasses and broad-leaved weeds. It was found to be a persistent environmental contaminant. Because of their polarity, triazine herbicides are usually not absorbed to the soil but percolate through it causing contamination of groundwater and surface waters. The methods generally used to measure pesticides and industrial pollutant residues are based on chromatographic techniques involving extraction and extensive purification procedures. Large sample volumes are often necessary to reach the required detection limits. Moreover, expensive and sophisticated equipment is needed, which must be run by experienced personnel. As a result, routine and efficient residue control by the official laboratories becomes problematic.

Food and environmental regulatory agencies have established control programs due to the increasing concern about the possibility that residues of those pesticides arrive to the consumer through food or through the contaminated environment. The European Community (EC), according to the Council Directives—86/362/EEC and 90/642/EEC—has thus established the maximum levels (MRL, maximum residue limits) for pesticide residues. Member States are requested to regularly check the levels of pesticides in foodstuffs. Inspections and monitoring procedures should be carried out in accordance with the provisions giving by the Council Directives 89/397/EEC 5 and 93/99/EC 6. Moreover, besides the national monitoring programs, the Commission Recommendation 2002/1/EC 9 requests the participation of each Member State in a specific EU coordinated monitoring program. According to these requirements, and in order to protect the public health, official laboratories should be able to efficiently process a high number of samples. As a consequence, the development of rapid, inexpensive, sensitive, and high sample throughput and on-site analytical strategies, which can be used as an “alarm” to rapidly detect the risk of contamination by pesticides of water resources and a wide variety of food matrices, such as fruit and fruit juices, vegetables, and other foodstuffs, are required.

Immunoassays (IAs) are analytical tests that use antibodies as specific recognition elements. The IA has proven to be one of the most productive technology contributions to medicine and fundamental life science research in the twentieth century for both
hand-mixed in a 1:4 (w/w) ratio. This mixture was thoroughly hand-mixed to ensure the uniform dispersion of the graphite powder throughout the polymer. The schematic representation of the construction of the m-GEC electrode is shown in Figure 1A. The resulting paste was placed to a depth of 3 mm in a cylindrical PVC sleeve body (6-mm i.d.) with a copper plate as electrical contact. For preparation of the m-GEC electrode, a small neodymium magnet (3-mm i.d.) was placed into the center of this electrode after the addition of a thin layer of composite paste in order to avoid direct contact between the magnet and the electrical connector (Figure 1A). After filling the electrode body gap completely with the soft paste, the electrode was tightly packed. The m-GEC electrodes were cured at 90°C for 3 days in order to obtain a rigid composite (Figure 1A). Before each use, the electrode surface of the m-GEC electrodes were renewed by a simple polishing procedure, wetted with double-distilled water, and then thoroughly smoothed with abrasive paper and then with alumina paper (polishing strips 301044-001, Orion).

**Antibody Binding to the Magnetic Beads.** Antibodies were covalently coupled to MP-COOH and MB-Tosyl magnetic beads while MB-ProtA magnetic beads were modified by affinity interactions trough the Fc part of the antibodies. The covalently modified magnetic beads were stable for at least three months if stored at 4°C, while MB-ProtAs were immediately used after the attachment of the antibody. Information of how the covalent or affinity modification of the antibodies was performed can be found in Supporting Information.

In all cases, three different pools of modified magnetic beads (MP-COOH, MB-Tosyl, MB-ProtA) were prepared: (i) with the specific Ab10 antibody; (ii) with the nonspecific antibody (Abpre); and (iii) without any antibody, only treated with the corresponding coupling buffer. The efficiency of the coupling strategies was evaluated by the Bradford test analyzing the protein concentration in the supernatant before and after the conjugation. The assay was performed by comparing the samples with a standard curve achieved with a reference IgG reagent.

**Competitive Assay Using a Magneto-ELISA Procedure.** Optimal concentrations of the enzymatic tracer and the antibody-modified magnetic beads were chosen to produce a signal ranging from 0.7 and 1 absorbance units in 30 min by performing a two-dimensional assay. Further experimental information is provided in Supporting Information.

The competitive assay was performed in microtiter plates, and all the referred quantities are “the amounts added per well”. After each incubation or washing step, the magnetic beads were separated from the supernatant on the side wall by using a magnet—positioned under the microtiter plate. Atrazine standard solutions (50 µL/well) in PBST (from 0.0032 to 1000 nmol L⁻¹)