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# Horia Hulubei National Institute for R&D in **Physics and Nuclear Engineering**

Process for preparing the enzimatic marker 3,6dichloro-2-methoxy benzoylhexamethylendiamin-glutharaldehyde-alkaline phosphatase

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#### **Abstract**

The invention relates to a process for preparing the enzymatic marker 3,6-dichloro-2-methoxybenzoylhexamethylendiamin-glutharaldehyd-alkaline

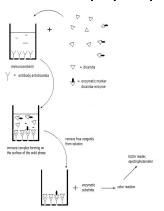
phosphatase, used in ELISA technique for determining the dosage of pesticide in biological and environmental samples. The obtained enzymatic marker pesticideenzyme has high affinity against antipesticide antibody and used in ELISA technique leads to a better sensitivity ELISA technique.

The pesticide 3,6 dichloro-2-methoxybenzoic acid (dicamba) is used in annual or perennial weed control development that may contaminate cereal crop production leading to their losses, protection of cereals, fruit (citrus, apples, pears, peaches etc) and in applications in the field of aquatic weed control in canals, reservoirs. Should be monitored because its presence in food products can produce toxic effects on ingestion, inhalation or dermal exposure in human organism and animals and teratogenic effects (malformations). Symptoms of poisoning with dicamba: loss of appetite (anorexia), vomiting, muscle weakness, effects on the heart and central nervous system (depression), cyanosis of skin, muscle spasms, irritant and corrosive can cause severe affections or permanent effects of eye. Dicamba affect plants as a result of absorption from soil by plant roots and its half-life in soil is between 1 and 6 weeks. It has high solubility in water and easily contaminate groundwater sources and through absorption in plants lead on possible contamination of crops, fruits and food.

ELISA (Enzyme Linked Immunosorbent Assay) is an immunochemical technique that uses antibodies, enzymatic markers and other reagents to analyze contaminant substances from alimentary food samples (immunoassay that use antibodies to measure the concentration of an analyte having a

high sensitivity to determine nanograms (10<sup>-9</sup> g), picogram (10<sup>-12</sup> g) or femtograms (10<sup>-15</sup>g) of contaminants from biological samples.

## **ELISA** technique principle



## **Technology stage**

The obtained product can be used in ELISA immunochemical technique for dosing the dicamba pesticide from environmental samples and it was validated in this technique.

### **Applications**

- ELISA kits for detection of the pesticide 3,6dichloro-2-methoxybenzoic acid (dicamba) from alimentary and environmental samples which leads to increased quality of life through use of uncontaminated food.
- Environmental protection: quantitative analysis of pesticide contaminants from environmental factors (soil, water).

# **Advantages**

- By using the hexamethylenediamine and glutaraldehyde the distance between coupled pesticide to enzyme is high compared to direct binding method of the carboxy group's pesticide to free amino groups of the enzyme leading to a higher affinity antibody against the enzymatic marker towards antipesticide antibody from ELISA method and respectively the better sensitivity of the technique.
- Is avoiding partial inactivation of the enzyme through reaction medium used by the direct method.

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