In Phase I, suitable antibodies against the pesticides atrazine and 2,4-D have been developed, characterized and tested for their specificity, sensitivity and cross-reactivity with other similar and interfering substances. They were labelled with colloidal gold particles (20 nm) and a fluorescent dye (FITC) for developing dipstick based immunodiagnostic kits. However, the limit of detection of pesticides using dipsticks reached only down to 50 ppb levels. Therefore, the sensitivity enhancement of lateral flow based immuno-dipstick kits was the prime objective of the second programme phase, with the aim of bringing the limit of detection of pesticides in real water samples down to 1 ppb level. In parallel, such dipstick methods are to be developed using chemiluminescent techniques and applied not only to atrazine and 2,4-D, but also to methyl and ethyl parathions. The earlier method of producing antibodies from rabbit serum (IgG) was extended to a new procedure by which good quality antibodies are produced from hen’s egg yolks (IgY). A parallel task was to establish a flow injection analytical system employing chemiluminescence method for the quantitative detection of pesticides. Another key scientific goal was to develop fluorescent quantum dots (Q-dots) and Q-dot bound conjugates to enable the development of a single analyte detection method, which will be developed further into a multiple analyte system at a later stage. The project relied on IMTECH for the development of gold nanoparticle based dipsticks and antibodies for atrazine and 2,4-D. CFTRI’s role was on the development of egg-yolk based antibodies of parathions and chemiluminescence based detection methods. Appropriate types of Q-dots and related conjugates were the tasks of EPFL. The consortium further intended to facilitate the commercial exploitation of their key findings by initiating collaboration with a suitable industrial partner, such that the society at large would benefit from this project with much needed practical applications.

All three partners were in close contact with each other throughout the project period and many experiments were jointly conducted. Not only samples, but also experimental protocols were exchanged amongst the three partners. As a result, the sensitivity of detection for atrazine by the dipstick method was brought down from >50 ppb level to 1 ppb level. This became possible by the use of a chemical signal enhancer based on gold nanoparticles. Further, these kits have been tested and validated for their use in field conditions. Chemiluminescence method was extended to different pesticides and herbicides by using different types of antibodies originating from both CFTRI and IMTECH. As a result of this approach, we now have both the dipstick and FIA (FITC???) versions of the biosensor devices. Together with gold NP based dipsticks, the BR2 project now offers the user an option to choose between different devices to suit their analytical requirements, which broadly can be listed as qualitative, quantitative, semi-quantitative, rapid and field applicable. Today, the consortium has the know-how to produce good quality IgG and IgY antibodies for ethyl and methyl parathions, 2,4-D and atrazine. It is possible to enlarge this list to other pesticides and herbicides by following similar...
production strategies. The project thus created a critical mass of expertise in India to sustain research and developmental activities in the field of biosensors.

The ability of Q-dots to function as reporter groups in assays open up a way for the development of multi-analyte assays. Appropriate protocols for the preparation of Q-dot conjugated hapten-protein conjugates have been developed. An immunoreactor column for Q-dot based detection system has been also developed. The progress so far shows that atrazine can be detected using Q-dot markers down to 10 ppt level, which is a significant achievement. The protocols developed for Q-dot markers can further be optimized to suit multi-analyte detection of different pesticides by a single analysis. With these results, it is now certain that in the next phase of BR2 project, where an industrial partner will also be involved, a multi-analyte assay meeting today’s market needs will be realized.

The project consortium has already identified a suitable industrial partner called Bigtec Pvt. Ltd., Bangalore, for proceeding with the transfer of know-how. At the end of this project, the present partners have already taken the resolve to proceed with the formulation of a new collaborative project with the participation of Bigtec Pvt. Ltd., Bangalore. The goal of the new project is to translate the scientific output of project BR2 into commercially viable state-of-the-art modular biosensor devices. The new industrial project is expected to commence by the end of 2008.